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EVALUATION OF ASSOCIATION BETWEEN OXIDATIVE STRESS PARAMETERS AND PESTICIDE EXPOSURE IN CASE OF RECURRENT PREGNANCY LOSS AMONG NORTH INDIAN POPULATION

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ABSTRACT

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% to 3% of pregnancies. RPL is a challenging medical problem because of its unknown pathogenesis and etiology in most of the cases. Environmental contaminant may adversely affect the human reproductive health includes heavy metals, pesticides and other agents.The present study was conducted to investigate possible associations of pesticide exposed oxidative stress in the pathogenesis of RPL. Seventy women with two or more consecutive pregnancy losses were enrolled along with healthy women having one or more healthy and live issue as controls (70). The complete plasma lipid peroxidation products, enzymatic and non-enzymatic antioxidants were measured according to respective protocols. The levels of antioxidant enzymes namely catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase were significantly reduced in cases. However, the level of lipid peroxides, protein carbonyls and conjugated dienes were found significantly increased in all patients who experienced recurrent pregnancy loss. On the basis of our results, it may be concluded that pesticide exposure tends to increase oxidative stress with the increase of pro-oxidant components, 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, Antioxidant, Pesticide.**

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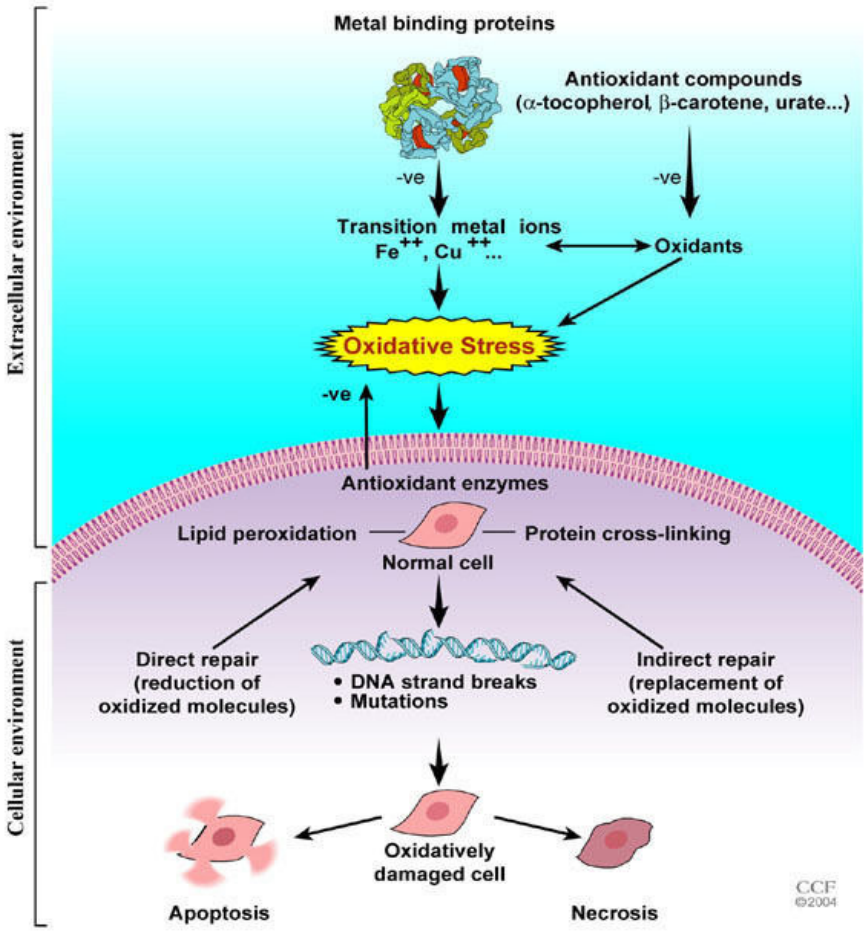
1. INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as a minimum of three consecutive miscarriages affects 1–3% of women[1,2]. The incidence of recurrent pregnancy loss should be approximately 1 in 300 pregnancies. However, epidemiologic studies have revealed that 1% to 2% of women experience recurrent pregnancy loss [3]. RPL is a challenging medical problem because of its least known pathogenesis and etiology in most of the cases. Both fetal and maternal factors are found to be associated with the pathophysiology of RPL. Fetal factors include genetic or developmental abnormalities, while uterine pathology, endocrine dysfunction, antiphospholipid syndrome and thrombophilic disorders have been identified as maternal aspects [4]. The role of other factors like infections, hormonal imbalances, parity, menstrual disorders, nutritional deficiencies, psychological trauma, stress life events, alcohol and caffeine intake have been studied earlier but the results are inconsistent [5,6]. More recently, environmental pollutants including pesticides have been implicated in the pathogenesis of reproductive disorders [7]. A positive association between smoking and occupational exposure to pesticides with increased risk of abortion / miscarriages has been reported in recent years [8,9]. In women with RPL, intensive diagnostics can identify the definite cause only in few cases [6]. Hence, further environmental factors such as must be identified that may affect pregnancy and play an etiological role in the pathogenesis of RPL. These environmental chemicals enters the body through oral, dermal and inhalation routes They are absorbed and distributed to different organs [10] and can cause damage and increase in oxidative stress. In a healthy body, ROS (reactive oxygen species) and antioxidants remain in balance. When the balance is disrupted towards an overabundance of ROS, oxidative stress (OS) occurs [11]. At higher levels, OS can cause indiscriminate damage to biological molecules, leading to loss of function and even cell death [12]. Most ROS are formed as a consequence of the mitochondrial respiratory chain, but can also be formed by exogenous exposures such as smoke and environmental pollutants [13]. Excessive ROS production, however, may overpower the body's natural antioxidant defense system, creating an environment unsuitable for normal female physiological reactions [14]. This, in turn, can lead to a number of reproductive diseases including endometriosis, polycystic ovary syndrome (PCOS), and unexplained infertility. It can also cause complications during pregnancy, such as spontaneous abortion, recurrent pregnancy loss (RPL), preeclampsia, and intrauterine growth restriction (IUGR) [15]. Pesticide chemicals may induce oxidative stress leading to generation of free radicals and alteration in antioxidants or oxygen free radical (OFR) scavenging enzyme system [16,17,18]. Lipid peroxidation has been suggested as one of the molecular mechanisms involved in pesticide-induced toxicity [19]. OFR enzymatic scavengers like superoxide dismutase (SOD), catalase (CAT), gamma-glutamyl transpeptidase (GGT), glutathione- S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR) etc., may protect the system from deleterious effect of OFRs [20, 21]. Oxidative stress plays an important role in the toxicity of various xenobiotics, including organophosphates (OPs), synthetic pyrethroid, organochlorine (OC) and carbamate pesticides [22]. The generation of free radicals is a normal physiological process but increased production of free radicals can act on lipids to cause lipid peroxidation. The cells have evolved a number of counter acting antioxidant defenses. Free radical scavenging mechanisms includes enzymatic and non-enzymatic antioxidants which limit the cellular concentration of free radicals and prevent excessive oxidative stress. The aim of the present study was to assess the markers of oxidative stress and antioxidative enzymes in pesticide exposed recurrent pregnancy loss.

Figure 1
Mechanisms of oxidative stress-induced cell damage (Adapted from A. Agrwal et al, 2005)[11]



2. MATERIALS AND METHODS

2.1. STUDY DESIGN AND SUBJECT

This was a case control study conducted at Queen Mary Hospital, King Georg's Medical University, Lucknow, UP, India. Informed consent was obtained from each subject. Present study was approved by the Institutional Ethical Committee of King George's Medical University, Lucknow, India. Seventy women (case) with a history of at least three recurrent pregnancy loss before the 20th week of gestation and attended Queen Mary Hospital, King Georg's Medical University, Lucknow, UP, India, from 2013 to 2015 were included in this study. An equal number of women (70) 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 syndrome, immunological causes of miscarriages, anemia, hypertension, bacterial vaginosis, TORCH infections, toxemia of pregnancy, 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. The spouses of these women were also non-diabetic with normal karyotype, normal sperm count and normal sperm morphology. The women we included in this study were of relatively homogenous group and they were similar in terms of demographical characteristics such as age, weight, BMI, food habits, drinking water supply, living style and socioeconomic status. We have excluded potentially confounding factors such as women of farming communities, occupational exposure to pesticides and industrial chemicals from this study.

2.2 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 were 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).

2.3 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) was assayed by the method of Pagila and Valentine [25]. The Glutathione reductase (GR) was assayed by the method of Hazelton and Lang [26]. Total protein contents of RBC sample were 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].

2.4 ANALYSIS

All the analysis were carried out on SPSS 16.0 version (Chicago, Inc., USA). The results are presented in mean±SD. Chi-square test were performed to compare categorical variables between cases and controls. The Unpaired t-test was used to compare discrete variables between cases and controls. The univariate and multivariate binary logistic regression was carried out to find the strength of the association of the study parameters. The unadjusted and adjusted odds ratio (OR) with its 95% confidence interval (CI) was calculated. The p-value<0.05 was considered significant.

3. RESULTS

3.1 DEMOGRAPHIC CHARECTERISATION

A total of 140 cases were enrolled in the present study. There were 70 subjects each in case and control group. Both groups were similar with respect to age, BMI, source of drinking water, and socioeconomic status was also almost similar. Table 1 shows the socio-demographic profile in cases and controls. There were no significant (p>0.05) difference in the mean age between cases and controls. All the socio-demographic variables were insignificantly (p>0.05) different between cases and controls.

Table. 1
Socio-demographic profile in cases and controls

	Cases (n=70)		**Controls (n=70)**		**p-value**
	No.	%	No.	%	
Age in years	28.53±5.25		27.70±5.18		0.34[a]
Place of residence					
Rural	27	38.6	25	35.7	0.72[b]
Urban	43	61.4	45	64.3	
Religion					
Hindu	58	82.9	54	77.1	0.39[b]
Muslim	12	17.1	16	22.9	
SES					
Higher	1	1.4	6	8.6	0.11[b]
Lower	30	42.9	32	45.7	
Middle	39	55.7	32	45.7	
BMI	25.86±1.69		25.81±1.79		0.86 [a]
Source of drinking water					
Filter	7	10.0	13	18.6	0.23[b]
Handpump	18	25.7	11	15.7	
Supply	41	58.6	44	62.9	
Well	4	5.7	2	2.9	
Food habit					
Non-Veg	28	40.0	24	34.3	0.48[b]
Veg	42	60.0	46	65.7	
Gestational age	2.36±0.51				

[a] Unpaired t-test, [b] Chi-square test

3.2 OXIDATIVE STRESS PARAMETERS

The comparison of oxidative stress parameters of both case and control group is summarized in Table 2. It was observed that the mean±SD values of blood level of antioxidant enzymes, SOD was 5.25±3.64 (U/mg protein), CAT was 25.79±18.79 (U/mg protein), GR was 0.52±0.32 (U/mg protein) and GPx 0.26±0.07 (U/mg protein) 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 4.19±3.17 (n mole MDA/mg protein), protein carbonyls0.09±0.05 (nmole/mg protein), and conjugated dienes 194.19±74.41 (µM) in cases and significantly decreased mean±SD values of blood level of LPO, PC and CD in control group.

Table. 2
Comparison of oxidative stress parameters in cases and controls

	Cases (n=70)	Controls (n=70)	p-value[1]
SOD (U/mg protein)	5.25±3.64	10.05±4.64	0.0001*
Catalase (U/mg protein)	25.79±18.79	52.09±22.98	0.0001*
LPO (nmole MDA/mg protein)	4.19±3.17	2.99±1.82	0.007*
GR (U/mg protein)	0.52±0.32	0.96±0.47	0.0001*
Protein carbonyl (nmole/mgprotein)	0.09±0.05	0.01±0.001	0.0001*
GPx (U/mg protein)	0.26±0.07	1.62±0.89	0.0001*
Conjugated dienes (μM)	194.19±74.41	130.67±60.51	0.0001*

[a]Unpaired t-test, *Significant

Table 3 shows the comparison of logistic regression analysis of all oxidative stress parameters in cases, revealed that among all oxidative stress parameters, SOD, CAT, LPO, GR, GPx and CD were significantly ($p<0.05$ or $p<0.01$) associated with the cases in univariate and multivariate analysis. However protein carbonyl was not significantly associated with cases in univariate and multivariate analysis.

Table. 3
Comparison of Strength of association of oxidative stress parameters for cases

	Unadjusted OR	95%CI	p-value[1]	Adjusted OR	95%CI	p-value[1]
SOD (U/mg protein)	0.75	0.67-0.83	0.0001*	0.69	0.58-0.81	0.0001*
Catalase(U/mg protein)	0.94	0.92-0.06	0.0001*	0.95	0.92-0.97	0.001*
LPO(nmole MDA/mg protein)	1.24	1.04-1.48	0.01*	1.30	1.03-1.65	0.02*
GR(U/mg protein)	0.06	0.02-0.18	0.0001*	0.03	0.004-0.17	0.0001*
Protein carbonyl(nmole/mgprotein)	1.01	0.11-2.19	0.12	1.01	0.10-2.11	0.13
GPx(U/mg protein)	0.46	0.11-0.87	0.0001*	0.39	0.09-0.79	0.001*
Conjugated dienes (μM)	1.01	1.01-1.02	0.0001*	1.02	1.01-1.03	0.001*

OR-Odds ratio, CI-Confidence interval, [1]Binary logistic regression, *Significant

4. DISSCUSION

The etiology of recurrent pregnancy loss is multifactorial and involves genetic and environmental factors like pesticide [31].Pesticide poisoning is primarily a problem of developing countries like India. Occupational exposure to toxic compounds, such as 'pesticides' (insecticides, herbicides and fungicides), is often cited as a risk factor for early pregnancy loss and pre-term delivery[32]. In this study, we have reported associations between gestational exposure to pesticide through dermal, inhalation and digestion route may cause oxidative stress in recurrent pregnancy loss that suggest increased attention, and reductions in lethargy, hypotonic responses, and signs of autonomic stress with higher exposure. In a study by Pathak et al (2003)[33], found significant difference between women with recurrent miscarriage (RM) and control subjects with respect to the blood concentration of γ-HCH organochlorine pesticide. Pesticide poisoning may induce oxidative stress leading to generation of free radicals. The excess production of free radicals and subsequent induction of OS, however, have long been known to significantly affect reproductive functions [11]. More recently, environmental pollutants including pesticides have been implicated in the pathogenesis of reproductive disorders[34,35] . Oxidative stress-induced damage has been hypothesized to play a role in spontaneous abortion and idiopathic recurrent pregnancy loss. When OS develops too early in pregnancy it can impair placental development and/or enhance syncytiotrophoblastic degeneration, culminating in pregnancy loss[36]. The syncytiotrophoblastic deterioration and OS that occur as a result of abnormal placentation may explain the heightened sensitivity of syncytiotrophoblasts to OS during the 1st trimester, and could contribute significantly to idiopathic RPL. In the present study, it was observed that the antioxidant enzymes, superoxide dismutase (SOD) and Catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), lipid peroxidation (LPO), protein carbonyl (PC) and conjugated dienes (CD) in pesticide exposed patients with recurrent pregnancy loss were significantly affected. We found significantly low level of SOD, CAT, GR and GPx in recurrent pregnancy loss as compared to control while MDA level was found to be increased in recurrent pregnancy loss as compared to control. Similar findings were reported in a study, they found significantly low levels of the antioxidant enzymes GPx, SOD, and catalase in patients with idiopathic RPL, in addition to increased MDA levels[37]. CAT and SOD are metaloproteins and accomplish their antioxidant function by enzymatically detoxifying the peroxides (–OOH), H_2O_2 and O_2 respectively. CAT has been suggested to provide an important pathway for H_2O_2 decomposition into H_2O and O_2. Oxidative stress, of which lipid peroxidation represents a major manifestation, is involved etiologically in a variety of clinical conditions including pregnancy and miscarriage[38]. Biochemical markers of ROS-induced membrane damage, such as lipid peroxidation products, reach high levels immediately before abortion. It has been proposed that an oxidant/antioxidant imbalance is associated with pregnancy loss. Oxidative stress has also been implicated as an important cause of recurrent

pregnancy loss[39]. Loss of antioxidant defenses have been shown to be associated with recurrent pregnancy loss [40]. We found higher level of conjugated dines in RPL similar finding were suggested in a study by Jozwik et al [41].

5. CONCLUSION

Present study concluded that pesticide exposed patients suffering from recurrent pregnancy loss were significantly affected by oxidative stress. This study has shown that the incidence and distribution of environmental exposure of pesticide as a cause of oxidative stress among couples with repeated fetal loss is comparable to that reported worldwide. Oxidative stress and ROS-induced damage may be the missing pieces of the puzzle of abortion and recurrent pregnancy loss of unexplained etiology. So, the risk of two consecutive losses can be reduced by assessing the reason through biochemical analysis of oxidative stress biomarkers, by giving antioxidant therapy and by avoiding the exposure of pesticide during pregnancy especially in early pregnancy. Variations in antioxidant levels have been documented and related to pregnancy loss, but there is a lack of consensus, and therapy with antioxidants is yet to be universally accepted. Tests for oxidative damage and counseling for avoidance to pesticide exposure should be considered as part of the diagnostic and treatment pathways for those suffering from recurrent pregnancy loss. 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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