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Health Problems and Reproductive outcomes of Women Agricultural Workers Exposed to Organophosphate Pesticides

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Abstract: *Organophosphate pesticides (OPs) are a group of toxic insecticides used in agriculture for protection against pests. They persist as environmental contaminants and pose significant risk to human health by causing respiratory system disorders, nervous system disorders, reproductive disorders and certain cancers. In the present study epidemiological and reproductive performance data of 500 women agricultural workers exposed to OP pesticides and 500 age and sex matched controls with same socio economic status was evaluated using standard questionnaire. Serum paraoxonase levels were analyzed by ELISA kit method. Statistical analysis revealed a significant increase in the frequency of abortions, stillbirths, neonatal deaths and congenital defects in the offspring of the exposed group when compared to the controls. There was a decrease in the frequency of fertile females and live births were observed in the exposed group when compared to the data from controls. Serum paraoxonase levels were also significantly low in the exposed group (7.61 ng/mL) when compared to control group (13.09ng/mL). The findings suggest that prolonged exposure to organophosphate pesticides with low serum PON1 levels may induce adverse health and reproductive problems in women agricultural workers.*

Keywords: *Organophosphate pesticides, Reproductive Epidemiology, Occupational Exposure, Environmental contaminants.*

I. INTRODUCTION

Human exposures to environmental and occupational chemicals have been increased considerably in the past 50 years. Pesticides are among the most produced and used chemicals all over the world. Organophosphate (OP) Pesticides have been considered as a boon for especially to the developing nations in their efforts to eradicate insect-borne endemic diseases and to increase production (1). OP pesticide poisoning is the major challenging public health problem in developing countries. Large amounts of these chemicals are released into the environment and many of them affect non-target organisms including humans (2).

OP pesticides can cause both acute and chronic health effects in humans. Acute health effects or short-term adverse health effects include stinging eyes, rashes, blisters, blindness, nausea, dizziness, diarrhea and death. Chronic health effects that can occur months or years after exposure are cancers, birth defects, reproductive problems, neurological and developmental toxicity, immune toxicity, and disruption of the endocrine system (3). Prolonged exposure to OP pesticides may pose a risk to genome integrity. Earlier studies on organ phosphorus pesticides have revealed induced apoptosis, necrosis and DNA damage in cultured human peripheral blood lymphocytes in in-vitro conditions (4,5). Juliana et al, 2008 (6) observed an elevated frequency of MN and DNA damage in OP pesticide-exposed vineyard workers in Brazil. Chromosomal aberrations like chromatid breaks and translocation frequencies were significantly high in OP pesticide manufactures (7).

OP Pesticides are also known to alter reproductive function by reducing brain acetyl cholinesterase activity and monoamine levels, thus impairing hypothalamic and/or pituitary endocrine functions and gonadal processes (8,9). The effect of exposure to OP pesticides during the reproductive cycle from preconception to breast feeding may possibly lead to poor birth outcomes, congenital anomalies and developmental deficits (10). Genetic damage occurring in the gonadal cells may result in the impairment of reproductive performance of exposed individuals and also contagious genetic defects that may appear in the future generations (11). Paraoxonase (PON1) enzyme was considered to be one of the most important biomarker of susceptibility to long term exposure to OP pesticides. Alterations in this serum concentrations and enzyme activity may contribute to inter individual variations in disease susceptibility (12-16). From previous animal studies it is evident that low or no serum PON-1 activity animals were found to be more sensitive to OP toxicity than high activity animals (17). Apart from polymorphisms PON-1 levels also play a major role in modulating the toxicity of OPs.

OP Pesticides have become integral part of villages in Guntur district of Andhra Pradesh and Nalgonda district of Telangana as rice, chilly and cotton belts are more common. Genetic bio-monitoring of populations exposed to potential carcinogens is an early warning system for genetic diseases or cancer. It also allows identification of risk factors at a time when control measures could still be implemented. To our knowledge there are hardly any studies on the effects of OP pesticides on the reproductive outcome of women exposed to organophosphates from South India. Hence, present study was conducted to evaluate general health and reproductive performance of women agricultural workers who were occupationally exposed to OP pesticides.

II. MATERIALS AND METHODS

In the present study 500 women agricultural workers (age range 18-45 years) with duration of service ranging from 2-22 years formed the exposed group. The working hours were 8-10 hrs per day. 500 women (age range 20 -45 years) belonging to same age and socioeconomic status and not exposed to OP pesticides formed the control group. All the subjects were clinically examined and data were collected using a standard questionnaire.

The questionnaire included general information (age, sex, education), occupational information (nature of work, duration of service, number of working hours), family history (marital status, consanguinity), reproductive history (number of live children, abortions, still births, neonatal deaths, congenital defects) and medical history (occurrence of chronic diseases, recent medication information, exposure to x-rays, etc.).

Blood samples were collected by venopuncture in plain tubes for serum. All the samples were processed within 12 hours. Blood samples were centrifuged to separate serum and stored at -80°C until analysis.

The study was approved by the Institutional Ethics Committee of the Institute of Genetics and Hospital for Genetic Diseases, Hyderabad. Verbal and written informed consent was obtained from all the study subjects involved after they were notified with the details concerning the aims of the research study.

A. Measurement Of Serum Paraoxonase Enzyme

Serum Paraoxonase (PON1) enzyme levels were assessed using enzyme-linked immunosorbent assay (ELISA) kit (Wuhan EIAab Science Co., Ltd, Catalog # EIA-E0243H, China) based on the sandwich principle and the detection range of the samples was 1.56-100 ng/mL. The absorbance of the samples was measured by using a microplate reader at 450nm. PON1 in the samples was then determined by comparing the O.D. of the samples to the standard curve using Master Plex software.

B. Statistical Analysis

The data is presented as the mean \pm SD and *p* values were calculated using students' paired t-test and chi-square test. A comparison of variables between two groups was performed using the one-way ANOVA. A 2-tailed *p*-value of <0.05 was considered to be significant.

III. RESULTS

The data on age, body mass index, work hours per day, education, duration of service are presented in table 1. The data in the table 2 represents the health problems documented in the workers and the control subjects. There was a significant increase in the frequencies of Head ache (24.8%), Eye irritation (34.8%), Skin allergies (31.6%), Irregular periods (25.4%), Backache (23.2%), Respiratory problems (22.6%), Joint pains (20.6%), Chest pain (13.4%) and Hyper salivation (11%) among exposed group when compared to control group (13.6%, 15.4%, 8.4%, 8.6%, 13%, 12.2%, 8%, 6.6%, & 3.4%). Hysterectomies were also significantly high in exposed women (25.2%) when compared to controls (7.2%).

The data in the table 3 represents the reproductive epidemiology recorded in the workers and control group. The data from the exposed subjects was compared with the data from the control subjects. There was a significant increase in the frequency of abortions (16.27%) and neonatal deaths (3.10%) among the exposed group when compared to the frequency of abortions (6.46%) and neonatal deaths (1.36%) recorded in the control subjects.

There was a significant decrease in the frequency of live births (77.51%) in the exposed group when compared to the controls (91.23%). Frequency of stillbirths (3.1%) and congenital defects (2.19) were also significantly high in the offspring of exposed workers when compared to the data from the controls (0.93% & 0.42%).

The serum paraoxonase enzyme levels were significantly low in exposed subjects when compared to control subjects. The mean serum levels were 7.61 ± 2.11 ng/ml in the exposed women as against 13.09 ± 3.80 ng/ml in control group (table 4).

IV. DISCUSSION

Organophosphate (OP) pesticide poisoning is a major challenging public-health problem in developing countries. Large amounts of these chemicals are released into the environment and many of them affect non-target organisms. WHO estimates that three million cases of OP poisoning occur worldwide, mostly in the developing countries(18). Studies on organophosphate exposure have revealed numerous health effects attributed mainly to acetyl cholinesterase inhibition (19-21). In the present study significantly increased frequencies of health problems like headache, nausea, back ache, skin allergies, respiratory problems, hyper salivation and irregular periods were observed in women agricultural workers occupationally exposed to OP pesticides than control subjects. Our results are in agreement with Sungur and Guven, 2001(22), who also reported hyper salivation, abdominal pain, respiratory distress and skeletal muscle fasciculation's in organophosphate insecticide poisoning cases.

Pesticides exposure have also been associated with menstrual cycle disturbances, reduced fertility, prolonged time-to-pregnancy, spontaneous abortions, stillbirths, developmental defects and congenital malformations (23,24). Weidner et al, 1998 (25) reported increased risk of cryptorchidism in sons of female gardeners exposed to organophosphate insecticide in Denmark. Jarnail et al, 2010 (26) observed significantly high Spontaneous abortions and premature births in individuals residing in areas affected by heavy metal and OP pesticide pollution.

Assessment of reproductive efficiency of humans exposed to a particular toxicant reveals the fatal toxicity of that particular toxicant(27). Studies on OP pesticide exposure effecting reproductive performance of occupationally exposed women are inadequate in India. In the present study our findings on reproductive performance in women agriculture workers showed significantly increased frequency of abortions, premature births, still births, neonatal deaths and congenital defects in OP exposed women when compared to controls. These results suggest an adverse effect of OP pesticides on the reproductive performance of agricultural workers. Furthermore in our study a high percentage of illiterate women were present in the OP pesticide exposed group, which means only a few of them can follow the guidelines for the safe usage and storage of pesticides.

Several earlier studies demonstrated the importance of paraoxonase (PON1) polymorphisms in the efficacy of OP detoxification and relevancy for human health risk assessment but only few investigations aimed to identify susceptible populations among groups that are occupationally exposed to OPs. Our study is one among them mainly aimed to assess paraoxonase and its risk with long term exposure to OP pesticides. It is hypothesized that individuals with low serum activity of this enzyme would be expected to have low ability to metabolize organophosphate compounds (28). Earlier studies on animals have also demonstrated the role of PON1 in decreasing the toxicity of organophosphate pesticides. Rats injected with PON1 showed increased resistance towards OPs than in controls (29). In PON1 knockout mice experiment with absence of PON1 activity, the sensitivity to OPs mediated toxicity was found to be increased (30-32).

Low serum PON1 levels can lead to diseases such as cancers and metabolic diseases in OP exposed populations (33,34). In the present investigation a wide variation in PON1 serum levels between exposed and control groups were observed. Serum paraoxonase levels were 1.65 folds lower in the exposed group when compared to control group. These results are in accordance with Maryam et al, 2014 (35) who reported low PON1 levels in Iranian workers occupationally exposed to organophosphates. Therefore the results of the present study suggests that low serum PON1 levels could alter the metabolism of organophosphate compounds in women agricultural workers leading to disease susceptibility.

V. CONCLUSION

The results obtained in the current study suggest that prolonged exposure to OP pesticides with low serum PON1 levels may result in adverse health and reproductive outcomes. Pesticide safety education and management strategies can reduce the effects caused by pesticide exposure as women agricultural workers are unaware of the health hazards of OP pesticide exposure. To eradicate the OP pesticides risks on reproductive health further bio monitoring studies are needed in women agricultural workers.

VI. ACKNOWLEDGEMENTS

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A. Declaration of interest

This study was funded by the Department of Biotechnology, Government of India.

The author(s) declare that they have no competing interests.

Characteristics	Agricultural workers n=500		Controls n=500		p value
Age (years)	n	%	N	%	
Mean \pm SD	35.65 \pm 7.91		37.55 \pm 7.39		0.327(F)
Range	18-45		20-45		
BMI (kg/m ²)					
Under weight (<18.5)	120	24	100	20	0.006** (χ^2)
Normal (18.5-24.9)	240	48	290	58	
Obese (>25.0)	140	28	110	22	
Diet					
Veg	90	18	102	20.4	0.377(χ^2)
Nonveg	410	82	398	79.5	
Education					
Literate	235	47	360	72	>0.001**(χ^2)
Illiterate	265	53	140	28	
Consanguineous marriage					
Yes	104	20.8	80	16	0.07(χ^2)
No	396	79.2	420	84	
Duration of exposure (yrs)	12.65 \pm 6.52				
Range	2-25 yrs				
Working hours per day(hrs)	8.32 \pm 1.16				
Range	6-10hrs				

Table 1 : General characteristics of women agricultural workers and controls

Values are mean \pm standard deviation *p<0.05 significant **P<0.01 highly significant

In women agricultural workers and controls

Health Problems	Agricultural workers (n=500) Number (%)	Controls (n=500) Number (%)	χ^2 value	p-value
Headache	124(24.8)	68(13.6)	19.5	0.001**
Hypertension	42(8.4)	33(6.6)	0.92	0.336
Nausea	62(12.4)	40(8)	4.81	0.028*
Dizziness	39(7.8)	22(4.4)	4.46	0.034*
Acidity	45(9)	33(6.6)	1.68	0.194
Loss of appetite	42(8.4)	18(3.6)	9.37	0.021*
Skin allergy	158(31.6)	42(8.4)	82.66	<0.001**
Eye irritation	174(34.8)	77(15.4)	49.02	<0.001**
Fatigue	57(11.4)	32(6.4)	7.10	0.007**
Respiratory problems	113(22.6)	61(12.2)	18.10	0.002**
Chest pain	67(13.4)	33(6.6)	12.11	0.005**
Backache	116(23.2)	65(13)	16.86	0.004**
Joint pains	103(20.6)	40(8)	31.37	<0.001**
Hyper salivation	55(11)	17(3.4)	20.49	<0.001**
Abdominal pain	67(13.4)	24(4.8)	21.33	0.003**
Irregular periods	127(25.4)	43(8.6)	48.82	<0.001**
Hysterectomies	126(25.2)	36(7.2)	58.35	<0.001**

*p<0.05 significant **P<0.01 highly significant

Table 3: Data on reproductive epidemiology of women agricultural workers and controls

Group	No. of females	No. of fertile females	No. of pregnancies	No of live births	No. of abortions	No. of Still Births	No. of neonatal deaths	No. of congenital defects	No. of premature Births
Agricultural Workers	500	411 (85)*	1094	848 (77.51)*	178 (16.27)*	34 (3.1)*	34 (3.1)*	24 (2.19)*	52 (4.75)*
Controls	500	459 (91.8)	1175	1072 (91.23)	76 (6.46)	11 (0.93)	16 (1.36)	5 (0.42)	30 (2.55)
χ^2 p value		0.01 **		0.01 **	0.01**	0.03 *	0.04 *	0.04 *	0.03*

*p<0.05 significant **P<0.01 highly significant

Table 4: Mean serum paraoxonase enzyme levels (ng/mL) in agricultural workers and controls

Serum Paraoxonase enzyme levels (ng/mL)	N	Mean±SD	p value
Controls	212	13.08±3.79	0.001**
Agricultural Workers	205	7.61±2.11	

Values are mean ± standard deviation**P<0.01 highly significant

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