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Oxidative stress and cholinesterase depression among farm workers occupationally exposed to pesticides in India

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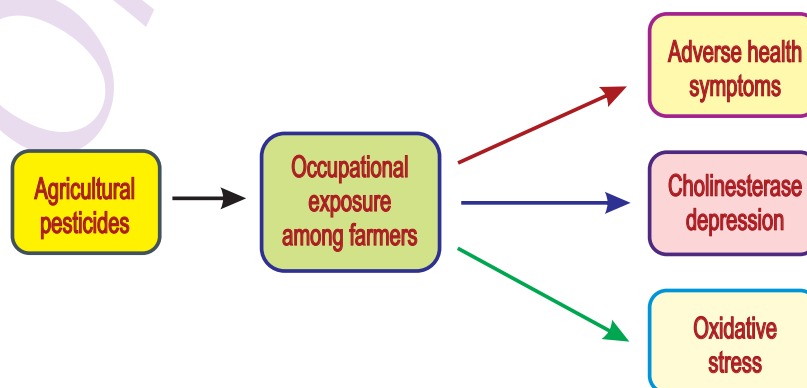
Abstract

Aim: This study aims to investigate the oxidative stress and cholinesterase levels among agricultural workers occupationally exposed to various agricultural pesticides.

Methodology: A cross sectional study was performed among fifty two male pesticide sprayers of Lucknow district in India, compared with thirty control subjects. Pesticide sprayers were selected on the basis of clinical examination for acute and chronic health symptoms associated with pesticide exposure.

Results: Clinical examination of pesticide sprayers showed significant health problems ($p < 0.05$) for different organ systems. Activities of acetylcholinesterase and butyrylcholinesterase were significantly depressed ($p < 0.05$) among pesticide sprayers as compared to controls showing the exposure to pesticides among them. Activity of blood CAT was higher but not statistically significant; activity of blood GPx was significantly higher ($p < 0.05$); blood GSH was significantly decreased ($p < 0.05$); blood MDA level was more in pesticide sprayers as compared to control subjects, but not statistically significant. These results show the sub-clinical state of oxidative stress among pesticide sprayers.

Interpretation: The present study showed that occupational exposure to pesticides leads to cholinesterase depression and alterations in anti-oxidant enzymes, eventually leading to oxidative stress condition.



Introduction

India is an agricultural country and pesticides are indiscriminately used for agricultural practices by farm workers in crop fields. India ranks 2nd in Asia and 12th globally in terms of annual pesticide consumption (Bhardwaj and Sharma, 2013). Unsafe methods of handling and spraying pesticides by farm workers in agricultural fields in India have led to occupational exposure to pesticides, which can cause hazardous health effects. In developing countries like India, pesticide sprayers do not use Personal Protective Equipments (PPE) like safety masks, gloves, aprons *etc.*, during spraying activities which result in their body exposure to pesticides *via* inhalation, ingestion and dermal absorption (Fareed *et al.*, 2013, Gupta, 2004).

Organophosphate pesticides inhibit the action of acetylcholinesterase which is an enzyme used to deactivate the nerve transmitter acetylcholine at nerve endings, thus Organophosphate pesticides increases the cholinergic effects of neurotransmitter, acetylcholine in the body and depolarization of neural transmission (Weiss *et al.*, 2004). Acetylcholinesterase activity (AChE) in the red blood cells and butyrylcholinesterase activity (BChE) in plasma have been used to monitor the extent of Organophosphate pesticides and carbamate exposure (Mekonnen and Ejigu, 2005). Gomes *et al.* (1997) observed inhibited AChE activity among the farm workers exposed to Organophosphate pesticides and carbamate pesticides. Decreased cholinesterase activity was observed among the farm workers who handled Organophosphate pesticides and carbamates (McConnell and Magnotti, 1994). Inhibited activity of BChE due to Organophosphate pesticides exposure was also observed among pesticide sprayers in India (Rastogi *et al.*, 2008). Both AChE and BChE level in blood is considered as important biomarkers for the extent of exposure to organophosphate and carbamate pesticides.

Oxidative stress plays an important role in toxicity of various pesticides including, synthetic pyrethroid, organochlorine and carbamate pesticides (Stevenson *et al.*, 1995). A balance between the production of free radicals and antioxidant defenses in the body has important health implications (Harman *et al.*, 1999). Pesticides may induce oxidative stress, leading to generation of free radicals and alteration in antioxidants, oxygen free radicals, the scavenging enzyme system, and lipid peroxidation (Banerjee *et al.*, 1999; Ahmed *et al.*, 2000; Banerjee *et al.*, 2001; Ranjbar *et al.*, 2002; Kesavachandran *et al.*, 2006; Singh *et al.*, 2007). Earlier studies have demonstrated oxidative stress induced by organophosphate pesticides in humans (Banerjee *et al.*, 2001; Ranjbar *et al.*, 2002).

Our research group previously reported respiratory health problems and alterations in hematological profile among pesticide sprayers who were exposed to organophosphate pesticides in rural agricultural sector of Lucknow

district in India (Fareed *et al.*, 2013). The aim of the present study was to explore cholinesterase depression and oxidative stress among the farm workers who are exposed to pesticides.

Materials and Methods

Study design : This is a comparative cross sectional study design which was conducted at Malihabad and Bakshi Ka Talab regions of Lucknow district located in North India. These areas have several mango plantations where agricultural workers spray pesticides to protect the mango crop from harmful pests thereby, enhancing the mango crop productivity. These agricultural workers have experience of spraying pesticides in mango plantation from 1–30 years. Spraying operations last for 2 to 8 hour / week per season on the trees of mango orchards from December to March every year. Mixing of chemicals with bare hands and leakage from tanks of pesticide during spraying operations were found to be common. Sprayers of mango plantations were using organophosphate pesticides (monocrotophos, dichlorvos, malathion, methyl parathion), organochlorines (endosulfan, thiodon), pyrethroids (cypermethrin) and carbamates (carbaryl).

Subjects : The subjects were selected on the basis of the results of a preliminary questionnaire specifically designed for the study. All the subjects were engaged in spraying of agricultural pesticides without using any PPE. Fifty two male pesticide sprayers, who reported some chronic and acute health symptoms for different organ systems, were selected. The study was compared with 30 healthy males, having no previous or current occupational exposure to pesticides, taken as control from nearby areas with same socio-economic status. On the basis of area of living, ethnicity of both groups of subjects was almost similar. The purpose of the study was explained to all the participants and their consent was obtained. All the health surveys and collection of blood samples of pesticide sprayers were performed during the end phase of pesticide application season in the month of March with time interval of 1-2 days after the last spraying operation.

Pesticide sprayers were selected on the basis of inclusion and exclusion criteria.

Inclusion criteria of pesticide sprayers (exposed subjects) included : Male pesticide sprayers with age group of 18-60 yrs; Pesticide sprayers must have at least six months previous experience in spraying operations in field; Pesticide sprayers having minimum 2 chronic or acute health symptoms for any organ system.

Inclusion criteria of control subjects include healthy male resident who did not handle pesticides and having similar socio economic status and age group of exposed subjects.

Questionnaire : A preliminary structured questionnaire was

designed to record the personal and occupational information along with pesticide exposure and health status of the pesticide sprayers. Each pesticide sprayer was interviewed based on questionnaire like general information about the name, age, smoking habit of individual pesticide sprayer; work practices of pesticide sprayers such as use of PPE, hygienic practices during loading and unloading processes were recorded.

Clinical examination : Clinical examination for adverse health effects was done by a medical expert and the symptoms for chronic or acute illness were recorded for every pesticide sprayer who participated in the study. The clinical examination comprised of medical history, general physical examination and detailed examination of different organ systems *viz.* nervous, respiratory, cardiovascular, gastro-intestinal, ocular, dermal and musculoskeletal. Signs and symptoms observed for each subject were entered in the questionnaire.

Ethical issues : Human ethical clearance was obtained from "Human Ethics Committee" of Indian Institute of Toxicology Research for collecting blood samples and clinical investigations of the participants. All the participants provided their written informed consent to participate in the study, which was approved by Institutional Human Ethics Committee. All the documents including questionnaires and consent forms of all the participants were safely kept for data analysis.

Blood sample collection : Approximately 3.0 ml of venous blood was collected in the heparinised glass vials as coded samples from both the control and sprayers. Samples were transported to the Laboratory of Epidemiology Division of Indian Institute of Toxicology Research, Lucknow, India in ice-cold condition immediately after collection. Investigation of biochemical parameters for oxidative stress and cholinesterase estimation were carried out on same day of blood collection.

Estimation of cholinesterase activity : Blood AChE and BChE activities were assayed by the method of by Ellman *et al.* (1961) as modified by Chambers and Chambers, (1989) by taking acetylthiocholine iodide and butyrylthiocholine iodide respectively as substrate and expressed as mmols hydrolyzed $\text{h}^{-1} \text{I}^{-1}$ blood (IU I^{-1}). The AChE and BChE activities were measured as a biomarker of organophosphate pesticides and carbamate pesticides exposure among study subjects.

Estimation of oxidative stress parameters : Catalase (CAT) activity in the hemolysate was determined by the method of Sinha (1972) using H_2O_2 as substrate and expressed as $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{g}^{-1} \text{Hb}$. Glutathione peroxidase (GPx) activity in the hemolysate was assayed using H_2O_2 as substrate in the presence of Glutathione (GSH) following the method of Rotruck *et al.* (1973) and expressed as mol GSH oxidized $\text{min}^{-1} \text{g}^{-1} \text{Hb}$. Hemoglobin content in the hemolysate was measured by the method of Drabkin and Austin (1932). The extent of lipid

peroxidation in the whole blood was assayed by measuring the formation of malondialdehyde (MDA) content (Stocks and Dormandy, 1971), and expressed as nmol MDA formed per ml of blood using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{cm}^{-1}$. Reduced GSH was estimated in the whole blood (Kuo *et al.*, 1983) and expressed as $\mu\text{mol ml}^{-1}$ blood.

Statistical analysis : Descriptive statistics were generated for demographic parameters in the control group and pesticide sprayers. Frequencies and percentages have been shown for all the categorical parameters. Unpaired student's t test was used to compare the mean values of the quantitative characteristics (demographic parameters, oxidative stress parameters and cholinesterase activity) between the control and exposed group. Chi square test was applied for comparing categorical outcomes (health symptoms). The criterion for significance was set at $p < 0.05$. All the statistical analysis were performed using EPI INFO 7.1.1.0 software (Centre for Disease Control and Prevention, Georgia, USA) and IBM SPSS Statistics version 20.

Results and Discussion

Mean age of pesticide sprayers was 37.99 ± 15.34 (range: 18 - 55) years; mean height of pesticide sprayers was 163.64 ± 6.33 (range: 155 - 170) cms; mean weight of pesticide sprayers was 51.87 ± 8.34 (range: 46-67) kgs; mean BMI of pesticide sprayers was 19.28 ± 2.68 (range: 18 - 23) kg m^{-2} ; mean exposure of pesticides among sprayers was 14.08 ± 10.51 (range: 1 - 20) years, respectively.

Table 1 shows information about different agricultural work practices followed by pesticide sprayers which are based on preliminary questionnaire designed for this study. Information about the place of pesticide storage, place for pesticide preparation and handling, personal protecting equipment and awareness during mixing/loading/spraying, fate of empty packages/containers of pesticide is shown in Table 1.

Self reported health symptoms observed among pesticide sprayers (Table 2) were related to different organ systems *viz.* nervous (48.95%), ocular (35.15%), respiratory (38.49%), cardio vascular (40.58%), gastro-intestinal (37.66%), musculo-skeletal (58.58%) and dermal (9.62%). These symptoms among pesticide sprayers were significant as compared to control subjects.

The biomarkers of organophosphate pesticides and carbamate pesticides exposure *viz.*, acetylcholinesterase and butyrylcholinesterase activity in blood among pesticide sprayers were analysed and the results are shown in Table 3. Activities of both acetylcholinesterase and butyrylcholinesterase were found to be significantly depressed ($p < 0.05$) among the pesticide sprayers as compared to control subjects.

The oxidative stress parameters *viz.* catalase,

Table 1 : Information about the agricultural work practices followed by pesticide sprayers

Variables	Pesticide sprayers (N = 52) n (%)
Place for pesticide storage	
Inside the house	24 (46.15)
Inside plantation areas	5 (9.6)
No storage	8 (15.38)
Tools storage room	15 (28.84)
Place for pesticide preparation and handling	
In home	10 (19.23)
In fields	24 (46.15)
Near pond	21 (40.38)
Handling of pesticide container by bare hands	52 (100)
Mixing of pesticide by bare hands	17 (32.69)
Mixing of pesticide by rod	6 (11.04)
Mixing by motor	31 (59.61)
Personal protecting equipment and awareness during mixing/loading/ spraying	
Use of gloves/goggles/apron	0
Cloths wet during spraying	36 (69.23)
Smoke while spraying	14 (26.92)
Eat in the break between spraying	23 (44.23)
Take bath after the end of spraying	44 (84.61)
Change clothes at the end of work shift	42 (80.76)
Wash contaminated clothes with family clothes	36 (69.23)
Any integrated pest management training taken	0
Fate of empty packages /containers of pesticide	
Buried / burned	0
Discarded near the river/canal/field	35 (67.30)
Use at home	11 (21.15)
Sold to scrap dealer	5 (9.61)
Used for some other work	5 (9.61)

Table 2 : System wise health problems among study subjects

Organ systems	Reported symptoms	Control subjects (n=30)n (%)	No. of pesticide sprayers (N=52)n (%)
Nervous System	dizziness, headache and convulsions	1 (3.33)	25 (48.95)*
Ocular System	Blurred vision, refractive error, watering of eyes, pain in eyes, red swollen eyes, itching and burning sensation in eyes	0 (0)	18 (35.15)*
Respiratory System	Dry cough, productive cough and wheezing	0 (0)	20 (38.49)*
Cardio vascular System	Pain in chest, tachycardia and hypertension	2 (6.66)	21 (40.58)*
Gastro intestinal System	Hyperacidity, pain in abdomen and constipation	2 (6.66)	19 (37.66)*
Musculo skeletal System	Stiffness in joints and muscles, body ache, weakness in limbs	0 (0)	30 (58.58)*
Dermal System	Burning sensation of skin and itching	0 (0)	5 (9.62)*

*p < 0.05

glutathione peroxidase, glutathione and malondialdehyde for extent of lipid peroxidation among the pesticide sprayers with adverse health symptoms were analyzed (Table 4). Activity of blood catalase enzyme ($\times 10^4 \mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{g}^{-1} \text{Hb}$) was found to be higher in pesticide sprayers as compared to control subjects, but were not found to be statistically significant. Activity of blood glutathione peroxidase enzyme ($\mu\text{mol GSH}$ oxidized $\text{min}^{-1} \text{g}^{-1} \text{Hb}$) was found to be significantly higher ($p < 0.05$) in pesticide sprayers as compared to control subjects. Blood glutathione level ($\mu\text{mol ml}^{-1}$ blood) decreased significantly

($p < 0.05$) in pesticide sprayers as compared to control subjects. Blood malondialdehyde (nmol ml^{-1} blood) level, as an end product of lipid peroxidation was found to be more in pesticide sprayers as compared to control subjects, but was found to be statistically insignificant.

Correlation between different oxidative stress parameters and cholinesterase activity for both AChE and BChE are shown in Table 5. There was significant negative correlation of AChE with CAT, GPx and MDA ($p < 0.01$) and positive significant correlation between AChE and GSH ($p < 0.01$). There was also

Table 3 : Cholinesterase activity (IU L⁻¹) in study subjects

Cholinesterase activity (IU L ⁻¹)	Control subjects(n=30)Mean ± SD	Pesticide sprayers(n=52)Mean ± SD
Acetylcholinesterase	559.38 ± 296.41	411.95 ± 218.73*
Butyrylcholinesterase	414.79 ± 178.92	270.97 ± 193.07*

*p < 0.05

Table 4 : Parameters for oxidative stress among study subjects

Parameters	Control subjects (n=30)Mean ± SD	Pesticide sprayers (n=52)Mean ± SD
Catalase (x10 ⁴ imol H ₂ O ₂ decomposed/min/g Hb)	37.74 ± 12.67	43.47 ± 25.23
Glutathione peroxidase (m mol GSH oxidized/min/g Hb)	125.92 ± 43.30	142.73 ± 30.52*
Glutathione (m mol ml ⁻¹ blood)	21.19 ± 5.16	18.37 ± 5.33*
Malondialdehyde (nmol ml ⁻¹ blood)	12.99 ± 4.95	14.72 ± 4.28

* p < 0.05

Table 5 : Correlation of oxidative stress and cholinesterase activity among pesticide sprayers

Parameters	Correlation coefficients	AChE	BChE
CAT	r value	-0.95	-0.77
	p value	p < 0.01	p < 0.01
GPx	r value	-0.90	-0.74
	p value	p < 0.01	p < 0.01
GSH	r value	0.95	0.88
	p value	p < 0.01	p < 0.01
MDA	r value	-0.94	-0.73
	p value	p < 0.01	p < 0.01

significant negative correlation of BChE with CAT, GPx and MDA (p < 0.01) and significant positive correlation between BChE and GSH (p < 0.01), respectively.

The present study shows that due to unsafe work practices like not using PPEs, the pesticides sprayers were exposed to pesticides which might lead to different health risks. Mixing of chemicals with bare hands, leakages from tanks of pesticide during spraying operations was common. Pesticide sprayers did not use any personal protective equipments during spraying operations. The exposure of the subjects can be through breathing since no face masks were used by the sprayers at work place. Sprayers used to eat food or drink water during spraying operation without washing hands thereby, consuming food contaminated with pesticides which was another route for exposure. Since proper protective clothes were not worn by sprayers at work place, hence they were also exposed via dermal route.

Clinical examination showed illness for nervous system, ocular system, respiratory system, gastro-intestinal system, musculo-skeletal system and dermal system among the pesticide sprayers which clearly depicts the adverse health effects among the pesticide sprayers due to occupational exposure of agricultural pesticides. Earlier, in a study carried out south India,

the signs and symptoms of illnesses due to exposure to pesticides among farmers were assessed and farmers reported acute signs and symptoms of excessive sweating, burning/stinging/itching of eyes, dry/sore throat, excessive salivation (Chitra *et al.*, 2006) which were also found in the present study. In an earlier study, green house workers exposed to pesticides in Turkey showed symptoms of fatigue, headache, changes in mood, irritation in the eye, blurring of vision, difficulty in breathing, pressure on the chest, coughing, mucous saliva, skin itch, scars, dizziness, depression, forgetfulness, memory disorders, sleeplessness nausea, vomiting, bleeding of nose and weight loss (Ergonen *et al.*, 2005); similar type of symptoms were also reported in the present study. Clinical findings in this study support similar kind of studies on occupational exposure to pesticides among farm workers (Chitra *et al.*, 2006; Kesavachandran *et al.*, 2006, Ergonen *et al.*, 2005, Ecobichon *et al.*, 1996, Azmi *et al.*, 2009).

Organophosphate pesticides and carbamate pesticides used by the pesticide sprayers in the present study are neurotoxic in nature. The evidence of organophosphate pesticides toxicity was observed from depeleted AChE (RBC cholinesterase) and BChE (Plasma cholinesterase) in the blood of pesticide sprayers. Exposure to large doses of organophosphate pesticides is required for significant AChE inhibition to occur (He *et al.*, 1999).

Results of the present study related to inhibition in cholinesterase activity among pesticide exposed farm workers are in agreement with the previous studies (Gomes *et al.*, 1997; Lopez-Carillo and Lopez-Cervantes, 1993; Coye *et al.*, 1986; McConnell *et al.*, 1992, Rastogi *et al.*, 2008).

Oxidative stress plays an important role in the toxicity of various pesticides including organophosphate pesticides, synthetic pyrethroid, organochlorine and carbamate pesticides (Stevenson *et al.*, 1995). In the present study, high activity of blood CAT and GPx; low level of blood GSH and high level of blood MDA (increased lipid peroxidation) were found, which was evident as sub-clinical state of oxidative stress among the pesticide sprayers. CAT, GPx, GSH and LPO were found to be significantly correlated with AChE and BChE which shows that oxidative stress was found to be more among those subjects in which AChE and BChE activities were depressed, which shows that organophosphate pesticides pesticide exposure causing oxidative stress since depressed activity of AChE and BChE is implicated to organophosphate pesticides pesticide exposure. There is evidence that sub-chronic exposure to malathion increased malondialdehyde (MDA) content and decreased reduced form of glutathione (GSH) levels; and depressed activities of catalase, glutathione peroxidase, glutathione reductase and glutathione S-transferase in the blood, suggesting that organophosphate pesticides may induce oxidative stress leading to generation of free radicals and alteration in anti-oxidants or oxidized free radicals scavenging enzymes (Ahmed *et al.*, 2000). This study demonstrated oxidative stress among the pesticides sprayers with depressed AChE activity, however another study (Shadnia *et al.*, 2005) showed no difference in LPO and AChE activity among study group exposed to organophosphate pesticides. Some previous similar kind of studies support the findings of this study related to oxidative stress (Singh *et al.*, 2007, Prakasam *et al.* 2001, Ogut *et al.* 2011, Akhgari *et al.*, 2003, Ranjbar *et al.*, 2002). Increased activity of GPx in the present study could be due to significant production of H₂O₂ in pesticide-induced toxicity (Samanta and Chainy, 1997). In previous study, glutathione levels were depressed by organophosphate pesticides pesticide exposure through a process that results in activation of more reactive oxygen species leading to enhanced lipid peroxidation (Abdollahi *et al.*, 2004).

Present study shows that pesticide sprayers are at higher risk to acquire toxic effects due to unsafe occupational work practices. The study concludes that occupational exposure to pesticides leads to cholinesterase depression and alteration in anti-oxidant enzymes, eventually leading to oxidative stress condition.

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