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Effect of Organophosphorus on Biochemical Parameters on Agricultural Workers of Mango Orchards

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ABSTRACT

Male agricultural workers in the age group of 20-57 years employed in manual pesticide mixing, formulation and spraying at mango orchards at Malihabad and Mal, the mango belt in the vicinity of Lucknow were studied for occupational health risks. A control group belonging to similar socio-economic status and never involved either in agricultural operations or pesticide handling (reference group n = 50) was taken randomly from the same area for comparison purpose. Our findings revealed a high morbidity (76.6%) among the exposed population in contrast to (20%) observed in the reference group. The chief morbidity pertained to neuro symptoms and gastrointestinal problems as a result of occupational exposure to pesticides. Biochemical studies revealed that the acetyl-cholinesterase (AChE) activity in the blood was significantly lowered in the sprayers compared to the levels found in controls ($p<0.01$). Also, malondialdehyde level was significantly elevated among the sprayers ($p<0.01$) thereby indicating oxidative stress among the sprayers.

Key words: Agricultural workers, pesticide, morbidity, oxidative stress, acetyl-cholinesterase

INTRODUCTION

India is basically an agricultural country. Nearly 70% of its population lives in rural areas. The primary occupation of the rural population is agriculture. A diverse group of agro-chemicals is indiscriminately sprayed by the farmers for the pest control to save the crops. These agro-chemicals are popularly known as pesticides which have been broadly classified into organo-phosphorus, organo-chlorine pesticides, carbamates and pyrethroids etc. The most commonly sprayed pesticides are Organophos Phorus (OP) insecticides which are very powerful neurotoxins resulting in neurotoxicity on acute or chronic exposure. The accidental exposure such as ingestion of OP pesticides in the case of suicidal attempts, a very common practice in rural area of our country may cause pesticide poisoning which may be fatal if unattended medically promptly resulting in mortality. The first case of pesticide poisoning occurred in Kerala in 1958 where in over 100 people died after consuming wheat flour contaminated with ethyl parathion known as Folidol E 605. Another major case of pesticide poisoning occurred during Bhopal gas leak (MIC) in 1984, killing thousands of people. The morbidity and the mortality prevalence studies (Kesavachandran *et al*., 2006; Rastogi *et al*., 2009) have shown that there is an alarming rise in the rate of pesticide

poisoning in rural India. OP pesticide self poisoning is an important clinical problem in rural regions of the developing world in including India that kills an estimated 200,000 people every year. Exposure to even small amounts of an OP compound can be fatal; death is usually caused by respiratory failure resulting from paralysis of diaphragm and intercostal muscles. Deliberate self poisoning has reached epidemic proportions in rural parts of India where the toxicity of available poisons and sparse medical facilities ensures a high fatality rate of 20% is common. The occupational and environmental exposure to pesticides is very high in the farming population and their families including children due to para-occupational exposure resulting from storage and dumping of these chemicals in their residential premises. It is, therefore the rural community which is at a greater health risk due to consistent use of the various pesticides. Earlier studies (Jevaratnam, 1990; Ferrer, 2003) have shown that the acute and chronic exposure of pesticides through inhalation, in gestation and dermal absorption may cause neurotoxicity (acute or delayed type) present a wide variety of symptoms resulting from the inhibition of secretion and formation of acetyl-choline-esterase enzyme by the pesticides directly. Due to non-formation of these enzymes there is no breakdown of acetylcholine at the parasympathetic postganglionic nerve endings resulting in excess accumulation of acetylcholine at the nerve endings causing cholinergic syndrome which is reflected in the form of nicotinic and muscaranic symptoms such as vagal inhibition, contraction of smooth muscles of bronchioles, meiosis, increased tear secretion and salivary secretion , increased GIT motility etc are some of the prominent clinical findings reported in the literature (Von Ostem *et al*., 2004; Hernandez *et al*., 2004). Some of the common earlier symptoms reported on acute exposure to pesticide are headache, nausea, giddiness, gastric pain etc. among the occupationally exposed agricultural workers (Wilson *et al*., 2005; Fuzikawa *et al*., 2005).

As the root cause of pesticide poisoning is the significant decrease in the formation of acetyl-choline-esterase enzyme and its activity is lowered by pesticide it is mandatory to measure the activity and its level in the affected cases. Bio-monitoring acetyl-choline-esterase is the fore-most important procedure in clinical management of pesticide poisoning. Some of the investigators (Hernandez *et al*., 2005) have recommended determination of acetyl-choline-esterase activity in the blood (in isolated RBC). The enzyme formed in RBC's is the "True" acetyl-choline-esterase while some studies (Anwar, 1997; Banerjee *et al*., 1999; Prakasam *et al*., 2001) preferred to estimate pseudo-acetyl-cholinesterase in plasma which is called as plasma butyryl choline-easterase (BuChE). It has been found that both the formats of the enzyme are equally sensitive biomarkers of pesticide poisoning. The present study was under taken to study the effect of occupational organophosphate pesticide exposure on neuro-physiological and gastrointestinal functions of pesticide sprayers engaged in mango plantation at Malihabad and Mall areas in the vicinity of Lucknow. 150 male pesticide sprayers in the age group of 20-57 years were randomly selected to the study.

MATERIALS AND METHODS

The proposed study selected randomly 150 OP pesticide exposed male rural agricultural workers in the age group of 20 to 57 years having at least one year's exposure to OP pesticides as sprayers in the mango orchards in and around Mal and Malihabad areas. A reference group (n = 50) belonging to similar socio-economic status from the same areas were selected as controls having no history of exposure to OP. The details of clothing worn and the protective devices, if used, are noted on a pre designed survey proforma. These pesticide sprayers normally and frequently sprayed insecticides to control pests of mangoes such as mealy bug, mango hoppers and mango scale which

cause a lot of damage during the flowering and fruiting seasons. Some of the very common Op pesticides sprayed in mango orchards are malathion, endosulfan, monochrotophus, methyl parathion, phosphomedon and their mixture in different combinations. Spraying normally starts at the end of December and continues until April/ May till the mango fruit ripened. The sprayers are thus exposed to aerosols of pesticides during aerial spraying as well as during manual mixing and loading. Pesticides application periods last for 4 to 5 hours per week and the spraying operation covers an average of 0.5 hectares area per day. The health examination was offered free of charge to the pesticide applicators and sprayers working in the mango plantations in Malihabad areas.

Determination of cholinesterase activity: Acetyl-cholinesterase and butyryl-cholinesterase activity in blood was estimated by the method of Elman *et al.* (1961) as modified by Chambers and Chambers (1989) by taking acetylcholine iodide as substrate and expressed as mmoles hydrolyzed/h/l blood (I U^{-1}). For assay, 0.025 mL of 25 fold diluted blood was mixed with 0.015 mL of diluting buffer (Tris-HCl, 100 mM, pH 7.4) containing 0.1 mM acetylcholine iodide. The reaction mixture was incubated for 15 min with constant shaking at 37°C and terminated with 0.5 mL mixture of DTNB and SDS (0.04 and 44%, respectively, in diluting buffer). The absorbance was read at 412 nm and converted to equivalent of mmoles hydrolyzed using molar extinction coefficient of 13600 mole L^{-1} cm^{-1}.

Estimation of glutathione: The level of GSH was estimated in the blood by the method of Jallow *et al.* (1974). 0.5 mL of blood was mixed with 1.5 mL of water, 2 mL of 10% TCA and centrifuged at 2000 rpm for 15 min. To the supernatant (1 mL), 4 mL 0.1 M phosphate buffer (pH 7.4) and 0.1 mL of 0.4% DTNB in phosphate buffer was added and the color was read at 412 nm.

Estimation of lipid peroxidation: Malondialdehyde (MDA) was estimated in the blood by the method Stocks and Dormandy (1971). 0.5 mL of blood in phosphate buffer (pH 7.4, 100 mM) was incubated for 30 min at 37°C and centrifuged. To the supernatant (3 mL) 1 mL 1% TBA was added and kept in boiling water bath for 15 min. Contents were cooled in ice water and centrifuged for 15 min at 2500 rpm. The absorbance was taken at 532 nm and converted to equivalent of MDA (nmol/mL blood) using molar extinction coefficient of 1.56×10^5 mol L^{-1} cm^{-1}.

RESULTS

The personal and occupational details of the controls and exposed workers are shown in Table 1. OP pesticides commonly sprayed by the workers are given in Table 2a-c. The morbidity profile in the exposed and control group is illustrated in Table 3 shows the prevalence of overall morbidity to be 76.6 vs. 20% in the exposed workers and the reference group, respectively. The major morbidity observed in the OP pesticide subjects was primarily neurological involving both central and peripheral nervous systems. Moreover, in this study the result showed that 6.6% exposed workers exhibited signs and symptoms of peripheral neuropathy. Out of 6.6% cases, half of them confirmed of affected by confirmed peripheral neuropathy resulting from OP pesticide. Other morbidities such as respiratory, ocular and Gastro-Intestinal Tract (GIT) were found to be quite prevalent in the exposed group as exposure to pesticides involves routes of inhalation, ingestion and dermal contact. The controls mainly suffered from GIT problems (Fig. 1).

Table 1: Sample size drawn from each area of the study with demographic details

	Exposed workers			
	Malihabad Area-1	Mall Area-2	Rahimabad Area-3	Control
Areas	Mean+S.D	Mean+S.D	Mean+S.D	Mean+S.D
Sample size	50	50	50	50
Mean age (years)	29.60+6.01	31.75+6.55	28.70+9.35	30.39+8.40
Smoking prevalence (%)	31(62%)	24(48%)	29(58%)	
Alcoholism prevalence (%)	24 (48%)	21(42%)	19(38%)	
Mean duration of exposure	12.5+4.8	16.2+6.4	13.6+5.6	
Use of personal protective devices (PPE)				
Hand gloves	-	-		-
Goggles	10%	15%	-	5%
Face mask	8.5%	5.5%	10%	-
Boots	2.5%	3.7%	10.5%	
BMI (kg m^{-2})	21.71+3.11	20.69+2.01	2.2%	
			22.16+3.01	

Table 2a: Organophosphorus pesticides sprayed by the sprayers in Malihabad area (Area-1)

Name of pesticide	Duration of spraying (h $week^{-1}$)	Frequency of spraying (times $week^{-1}$)
Dichlorvos	8-10	2 to 3
Methyl parathion	6-8	1 to 2
Chlorpyrifos	8-10	2 to 4
Monocrotophos	8-10	2 to 3
Ethion	6-8	2 to 4

Table 2b: OP pesticides sprayed in mango orchards in Mall area (Area-2)

Name of pesticide	Duration of spraying (h $week^{-1}$)	Frequency of spraying (times $week^{-1}$)
Diazinon	10-12	3 to 4
Quinalphos	8-10	2 to 4
Profenofos	6-8	2 to 3
Phorate	10-14	2 to 4
Triazophos	8-10	3 to 5

Table 2c: OP pesticides sprayed in mango orchards in Rahimabad (Area-3)

Name of pesticide	Duration of spraying (h $week^{-1}$)	Frequency of spraying (times $week^{-1}$)
Monochrotophos	10-14	3 to 4
Malathion	8-10	2 to 4
Propetamphos	6-8	1 to 3
Trichlorofon	6-8	2 to 4
Fenitrothion	6-10	2 to 4
Fenthion	6-10	1 to 3

The age wise distribution of morbidity pattern is shown in Table 4. The prevalence of different diseases showed increasing trend with respect to age. The results indicated higher incidence of neurological diseases in >30 year age group in contrast to sample of <30 year age exposed to pesticide. The subjects suffered more in >0 years age group (10.7 vs. 3.5%). Other diseases such as gastro intestinal tract, cutaneous, ocular, musculo-skeletal did not show significant differences

Table 3: Prevalence of diseases in exposed and control groups

	Pesticide exposed workers (N = 150)		Controls (N = 50)	
Disease profile	n	%	n	%
Neurological	40	26.6	-	-
Peripheral neuropathy	10	6.6	-	-
GIT (Gastro-intestinal Tract)	18	12	4	8.0
Respiratory	15	10	2	4.0
Cutaneous	10	6.6	1	2.0
Ocular	9	6.6	1	2.0
CVS (Cardio vascular system)	8	5.3	1	2.0
Musculo-skeletal	5	3.3	1	2.0
Total morbidity	115	76.6	10	20.0

Table 4: Age wise distribution of diseases in the exposed and control groups

	Exposed group				Control group			
	<30 Yaers (N = 85)		>30 Yaers (N = 65)		<30 Years		>30 Years	
Disease profile	n	%	n	%	n	%	n	%
Neurological	19	22.31	21	32.3	-	-	-	-
Peripheral neuropathy	3	3.5	7	10.7	-	-	-	-
GIT	10	11.7	8	12.3	1	4.3	3	11.3
Respiratory	8	9.4	7	10.7	1	4.3	1	3.7
Cutaneous	6	7.0	4	6.1	0	-	1	3.7
Ocular	5	5.8	4	6.1	0	-	1	3.7
CVS	4	4.7	4	6.1	0	-	1	3.7
Musculoskeletal	4	4.7	1	1.5	1	4.3	0	
Overall disease profile	59	69.4	56	86.1	3	13.0	7	25.9

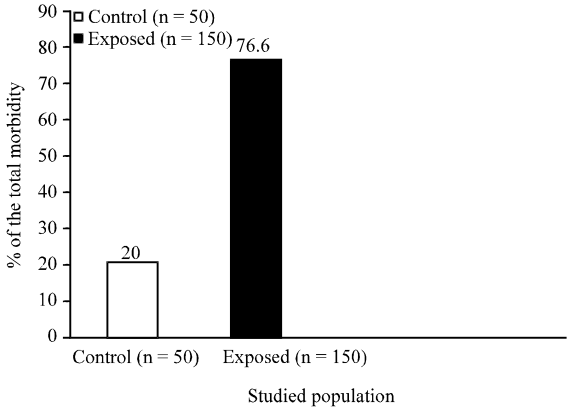

Fig. 1: Prevalence of disease in control and exposed subjects, Controls (N = 50) = 20.0%, Pesticide exposed workers (N = 150) = 76.6%

between the two groups although the overall prevalence of diseases was found to be significantly higher in senior age group (86.1 vs. 69.4%; $p<0.05$) (Fig. 2).

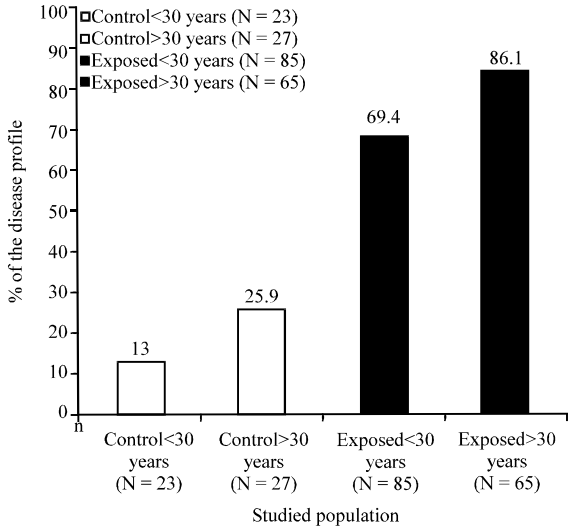

Fig. 2: Age wise distribution of disease in the exposed and control subjects, Control<30 years (N = 23) = 13.0%; Control>30 years (N = 27) = 25.9%, Exposed<30 years (N = 85) = 69.4%; Exposed>30 years (N = 65) = 86.1%

DISCUSSION

There is compelling evidence from the results of the study that increased serum level of lipid peroxides i.e., Malondialdehyde (MDA) was found in the pesticide exposed population compared to the corresponding value estimated in the control group. The increased values of MDA were maintained in relation to period of exposure to the different mixtures of organophosphorus pesticide pesticides sprayed in the mango orchard in the three areas of the study (Mall, Malihabad and Rahimabad) from where the pesticide exposed population sample was drawn. The significant increase in MDA activity was found to be associated with considerable fall in the activity of glutathione (GSH) enzyme ($p<0.001$). The elevated level of TBARS observed in this research could be due to decreased antioxidant activity caused by exposure to pesticide mixtures which result in an increased peroxidation of red cell membranes. In blood, normal erythrocyte function depends on an intact cell membrane which is the target for many toxic OP pesticides. It has been reported in pesticide exposed humans that these enzymes (MDA and GSH) associated with the antioxidant defence mechanisms change under the influence of pesticides. These enzymes efficiently scavenge toxic free radicals and are partly responsible for protection against lipid peroxidation due to OP pesticide exposure (Banerjee *et al*., 1999; Ranjbar *et al*., 2002b). The correlation between TBARS and AChE activity found in the present study is in agreement with the results obtained by other workers (Akhgari *et al*., 2003; Singh *et al*., 2007). The profile of the antioxidant enzymes (MDA, GSH) in different stages of neurotoxicity, starting from sub-acute to chronic neurotoxicity revealed decreased total antioxidant capacity (TAC and total thiol groups) and increase in thiobarbituric reactive substances supporting earlier findings (Ranjbar *et al*., 2002a; Handy *et al*., 2002; Nasntiet *et al*., 2003). It has been strongly suggested that in long-lasting exposure to OPIs, Reactive Oxygen Species (ROS) simply consume and exhaust antioxidant agent present in the body which is the reason why lower antioxidant capacity exists in chronic pesticide exposure while in sub-chronic exposure, the body is capable with the persistent OPI-induced reactive oxygen species in a longer period (Halliwell, 1994; Stohs, 1995; Abdollahi *et al*., 2004). The result of present study

indicate that glutathione (GSH) linked enzymes involved in cellular antioxidant defence system in human erythrocytes were significantly affected (significant decrease in enzyme activity) following exposure to mixtures of OP pesticides particularly in acute and chronic pesticide intoxication cases.

The RBC AChE activities were significantly reduced in the pesticide exposed workers compared to the control values and further data analysis showed that there is a maximum fall in activities of these enzymes. Different studies have recognized the invaluable role of AChE and BChE monitoring in rural agricultural workers at high risk for exposure to OP pesticides (McCanley *et al.*, 2006). It was observed that the blood cholinesterase activity needs at least a 15% decrease from an individual's normal level of plasma or erythrocyte enzyme activity to be considered indicative of pesticide over exposure. The acute and the chronic OP toxicity observed in this study was associated with the significant inhibition of AChE enzymes in the normal function of the nervous system which results in the accumulation of acetylcholine (ACh) in the synaptic gap leading to disruption of the nervous Cholinesterase activity has been one of the most important end points in assessing the exposure to OP pesticides. Most occupational exposure to OP pesticides occurs from skin absorption, although inhalation may be an important route of exposure during pesticide manufacture and application. Skin absorption can occur when dermal contact is made during handling and application of the insecticides, as most of the farm workers never use any Personal Protective Equipment (PPE) but prefer to work with bare bodies system (Domingues *et al.*, 2010). RBC AChE regeneration is restored only as new red blood cells are formed (Regeneration of red blood cells takes placed at a rate of about b1% per day). Most OPs need activation within the body to become anticholinergic compounds and there are differences between individuals in their ability to both activate and detoxify Ops.

CONCLUSION

Measurement of biochemical parameters in the blood of applicators suggested that their exposure to pesticides may contribute to decrease in AChE activity and increase in MDA both surrogate measurements for adverse effects from pesticide exposure. The use of pesticides in mango production is an important input by farmers. However, pesticide use may also present health concern to those making the application. Result of the study show that pesticide applicators complained of gastrointestinal, dermal and respiratory problems. The study based on the finding suggests selective and efficient use of protective measure (use of personal protective equipments and to conduct more awareness programs for rural community.

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