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Original Article

Assessment of Serum Cholinesterase in Rural Punjabi Sprayers Exposed to a Mixture of Pesticides

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ABSTRACT

Serum cholinesterase (SChE) activity is considered as a biomarker and is also taken as an exposure index to assess the low level, chronic residue exposures among sprayers. Thus, cholinesterase activity was studied in the professional rural Punjabi sprayers of Bathinda district in Punjab. This study was made to estimate the irregularities in the level of cholinesterase according to multiple pesticides used by sprayers, exposure periods, age, and body mass index (BMI) of the sprayers. The data generated was statistically analyzed by applying Student's '*t*' test and one-way analysis of variance. A positive correlation was found between SChE activity and years of exposure and a significant reduction in SChE activity was observed in younger population. Again, a positive correlation was seen between BMI and SChE inhibition.

Key words: Cholinesterase, pesticides, spray workers

INTRODUCTION

Pesticides constitute a heterogeneous category of chemicals specifically designed for the control of pests.[1] In recent years, the use of pesticides in agriculture has been increasing steadily, and at present, there are more than 1,000 chemicals classified as pesticides. In India, 15-20% of the total harvest is destroyed by pests resulting in uncontrolled use of pesticides by Indian cultivators.[2] The condition is quite grim in Punjab as the farmers spray five to six times more pesticides than recommended by Punjab Agricultural University.[3]

Farm workers/sprayers come into direct contact with pesticides and occupational exposure occurs during the preparations of the mixtures, loading and/or washing equipments, and spraying on crop.[4] Moreover, in most developing nations, safety equipment is rarely used and in some cases, completely lacking; storage methods are unsafe and instruction for pesticide use are not always understood since most farmers or pesticide users are uneducated, hence, increased risk of exposure.[5]

The principal classes of compounds used as insecticides are organochlorines, organophosphates (OP), carbamates, pyrethroid compounds, and various inorganic compounds. The chlorinated pesticides persist in the environment for very long periods, undergo bioaccumulation, and biomagnification and, therefore, impart toxicity to non-target organisms including human beings.[6] Toxic effects of OP and carbamate compounds are predominantly produced through inhibition of acetylcholinesterase (AChE), causing accumulation of acetylcholine at peripheral and central cholinergic receptors, resulting in overstimulation of the cholinergic system[7] and subsequent paralysis. The two cholinesterase enzymes capable of hydrolyzing acetylcholine in humans are acetylcholinesterase I (E.C. 3.1.1.7) found in erythrocytes, nerve endings, lung and spleen, and butyrylcholinesterase or acetyl

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cholinesterase II (E.C.3.1.1.8) which is found in serum, pancreas, and liver.

Exposure to even very small amount of an OP compound can be fatal; death is usually caused by respiratory failure resulting from paralysis of the diaphragm and intercostal muscles, depression of the brain respiratory center, bronchospasm, and excessive bronchial secretion.[8] OP and carbamate pesticides depress level of AChE, which can lead to neurological impairment. Serum Cholinesterase (SBChE) activities are taken as effective biomarker for monitoring pesticide exposure in farm workers.[9] Some researchers consider SBChE to be the exposure index to assess low level chronic residue exposures among sprayers.[10]

Thus this research work has been designed to study the effect of cocktail of pesticides on SBChE activity in rural sprayers of District Bathinda, Punjab.

MATERIALS AND METHODS

A total of 283 subjects, out of which 183 spray workers were exposed since childhood to a mixture of pesticides and 100 controls from same geographical area, who had no history of exposure to chemicals or other genotoxic substances from Bathinda district of Punjab Province, were randomly selected for this study. These spray workers were being exposed to a mixture of various types of pesticides during pesticide spraying season on different crops. Most of the spray work was done without using any protective measures like caps, gloves, full sleeve shirts, face masks, and shoes etc., Control subjects were not directly exposed to pesticides. These included school teachers, businessmen, shopkeepers, and other government employees not engaged in agriculture profession.

The sprayers were divided into various categories on the basis of pesticides used i.e., exposure time, total exposure period, age, and body mass index (BMI). To observe the activity of cholinesterase, further following subcategories were made (i) ≤5 years of exposure, (ii) 5-10 years exposure, and (iii) ≥10 years of exposure; (a) during spray, (b) within 1-10 days of spraying, (c) 10-25 days, and (d) ≥25 days of spraying; Age (1) ≤25 years, (2) 25-35 years, (3) 35-45 years, and (4) 45-60 years; and BMI (A) ≤18.5, (B) 18.6-25, and (C) 25.1-30. The most commonly used pesticides were categorized as (i) Monocrotophos, (ii) Chlorpyrifos, (iii) Profenofos, (iv) Acephate, (v) 2,2-dichlorovinyl dimethyl phosphate (DDVP), (vi) Endosulfan, (vii) Rift, (viii) Confidor, (ix) Acetamiprid, (x) Round up, (xi) Matador, (xii) Topic, and (xiii) Leader.

All the participants signed a written consent and answered a standard questionnaire regarding the demographic (age, gender etc.,) as well as the questions pertaining to medical history (any particular disease, vaccination, medication etc), smoking habit, and occupational exposure (years of exposure).

Blood samples (5 ml) were collected from cubital vein of all the participants with the help of a trained medical technician using sterile disposable syringe. Blood was left as such in syringe. The samples were kept in an ice box and were brought to the laboratory for making serum by centrifugation. Serum was made by centrifuging at 2,000-3,000 rpm for 15-20 minutes and was kept in well cleaned and labeled plastic vials for enzymatic analysis.

SBChE concentration was determined by a diagnostic kit, provided by Zilva and Pannall (1979)[11] procured from Randox Laboratories.

The data obtained was finally analyzed statistically using Student's *t*-test and analysis of variance (ANOVA). Values less than $P < 0.05$ were considered significant. The present research work has been carried out according to the guidelines issued by the Institutional human ethical committee.

RESULTS AND DISCUSSION

The mean ± S.D. activity of SChE in control group and various sprayers exposed to different pesticides is shown in Table 1. These values were found to be statistically

Table 1: Comparison of cholinesterase according to categories of pesticides sprayed by spray workers with control

	Cholinesterase			% age change	*t* value mean
	Mean	S.D	S.E.M		
Control (*n*=100)	7551.71	2013.73	201.37		
Spray workers (*n*=183)					
Organophosphate					
Monocrotophos (*n*=48)	4575.19	2145.58	309.69	−39.41	8.24***
Chlorpyrifos (*n*=17)	4788.00	1580.61	383.35	−36.59	5.37***
Profenofos (*n*=15)	5648.87	2129.38	549.30	−25.19	3.38***
Acephate (*n*=11)	6858.55	1355.63	408.74	−09.17	1.19$^{N.S}$
DDVP (*n*=09)	4628.11	1249.53	416.64	−38.71	4.27***
Organochlorine					
Endosulfan (*n*=05)	6464.60	1803.24	806.43	−14.39	1.18$^{N.S}$
Rift (*n*=04)	3666.25	1080.63	540.31	−51.45	3.82***
Neonicotinoid					
Confidor (*n*=74)	6059.57	1893.83	220.15	−19.75	4.95***
Acetamipride powder (*n*=20)	6851.10	1359.26	303.94	−9.27	1.48$^{N.S}$
Phosphanoglycine					
Round up (*n*=04)	3321.00	1083.77	544.89	−56.02	4.16***
Pyrethroid					
Matador (*n*=04)	6715.50	1130.85	565.43	−11.07	0.82$^{N.S}$
Topic (*n*=17)	6813.65	1395.13	338.37	−9.77	1.45$^{N.S}$
Leader (*n*=04)	9240.50	215.08	107.08	22.36	1.66$^{N.S}$

S.D.=Standard deviation, S.E.M=Standard error mean, '*t*'=Student's *t* test, ***Significant at *P*<0.0001, N.S.=Non significant

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significant ($P < 0.0001$) in categories i, ii, iii, v, vii, viii, ix, and x of pesticides but were non-significant ($P > 0.05$) in categories (iii, v, viii, xi, xii, and xiii) in comparison to control group.

The ANOVA values of SChE for exposed categories and unexposed population were also observed to be significant ($P < 0.0001$) as shown in Table 2.

To evaluate the effects of pesticides according to exposure time, the mean value of control was compared with mean values of pesticide exposed categories (i, ii, and iii). The mean values of SChE in pesticide exposed categories showed significant decrease ($P < 0.0001$) in all categories in comparison to control group [Figure 1]. Table 3 shows the values of SChE of control and spray workers according to exposure time, which was also found to be significant ($P < 0.0001$).

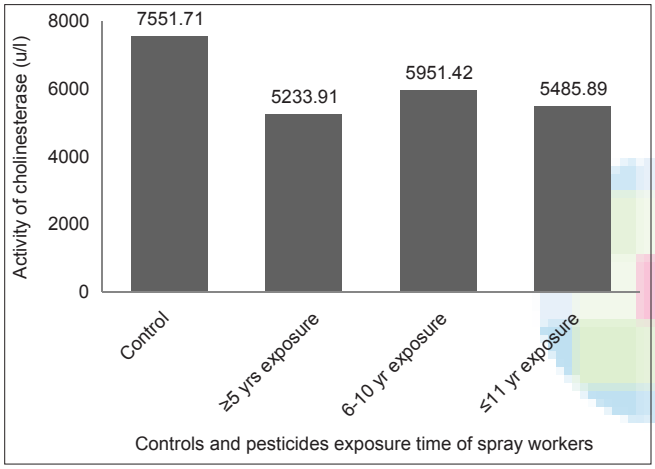


Figure 1: Comparison of cholinesterase according to pesticide exposure time of spray workers with control

Table 2: Analysis of variance for cholinesterase according to categories of pesticides sprayed by spray workers with control

Source of variation	SS	df	MS	F
Between groups	4.86E+08	13	37381822	10.76***
Within groups	1.1E+09	318	3472410	
Total	1.59E+09	331		

SS=Sum of Squares, F=One way ANOVA test, MS=Mean of squares, df=Degree of freedom, ***Significant at $P<0.0001$

Table 3: Analysis of variance for cholinesterase according to pesticide exposure time of spray workers with control

Source of variation	SS	df	MS	F
Between groups	266824521	3	88941507	21.72***
Within groups	1142416278	279	4094682	
Total	1409240799	282		

***Significant at $P<0.0001$, SS=Sum of squares, df=Degree of freedom, MS=Mean of squares, F=One way ANOVA test

In subcategories (a, b, c, and d), the activities of SChE showed significant reduction ($P < 0.0001$) in comparison to control group [Figure 2]. The results of SChE for exposed categories and unexposed population is given in Table 4, which were again found to be significant ($P < 0.0001$).

Significant reduction ($P < 0.0001$) in SChE concentration was found in all the exposed categories (1, 2, 3, and 4) in comparison to control group [Figure 3]. As shown in Table 5, significant variations ($P < 0.0001$) in concentration of SChE in all categories of spray workers and control were observed.

The values of SChE for exposed categories and unexposed population according to BMI are given in Table 6 and Figure 4 and these were again found to be significant ($P < 0.0001$) in all categories.

In present study, the depression in cholinesterase activity was found to be quite significant in each category of sprayers. As these occupational sprayers face acute exposure to a mixture

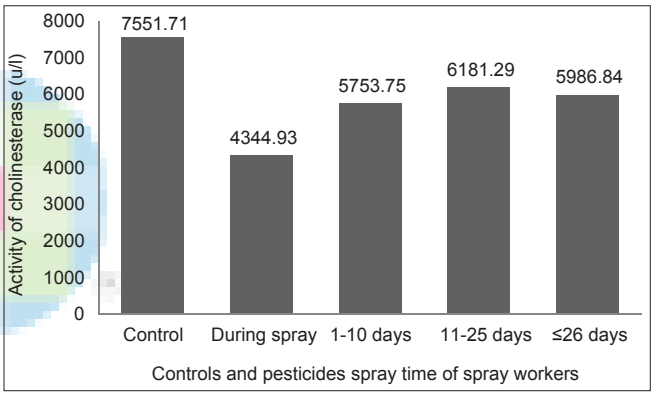


Figure 2: Comparison of cholinesterase according to pesticide spray time of spray workers with control

Table 4: Analysis of variance for cholinesterase according to pesticide spray time of spray workers with control

Source of variation	SS	df	MS	F
Between groups	337293416	4	84323354	21.74***
Within groups	1077799328	278	3876976	
Total	1415092744	282		

***Significant at $P<0.0001$, SS=Sum of squares, df=Degree of freedom, MS=Mean of squares, F=One way ANOVA test

Table 5: Analysis of variance for cholinesterase according to age of spray workers with control

Source of variation	SS	df	MS	F
Between groups	27673104	4	69093276	16.86***
Within groups	1138719414	278	4096113	
Total	1166392518	282		

*** Significant at $P<0.0001$, SS=Sum of squares, df=Degree of freedom, MS=Mean of squares, F=One way ANOVA test

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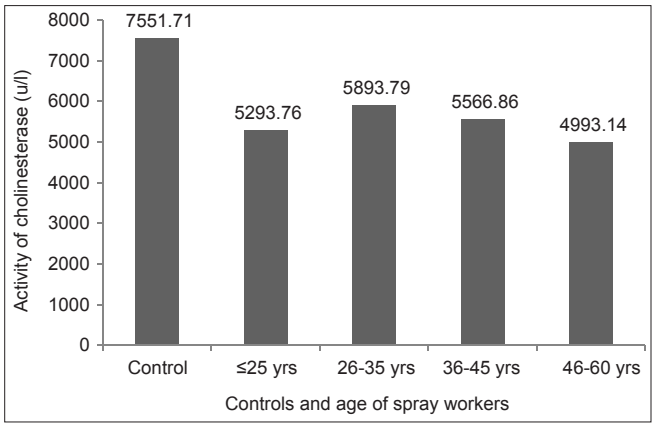


Figure 3: Comparison of cholinesterase according to age of spray workers with control

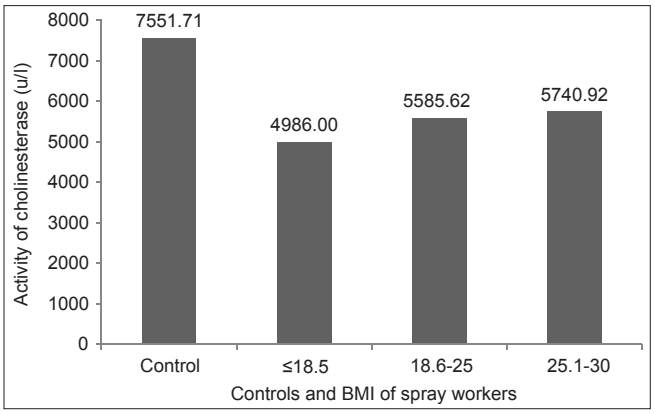


Figure 4: Comparison of cholinesterase according to body mass index of spray workers with control

Table 6: Analysis of variance for cholinesterase according to body mass index of spray workers with control

Source of variation	SS	df	MS	F
Between groups	265488744	3	88496248	21.47***
Within groups	1149603876	279	4120444	
Total	1415092620	282		

*** Significant at *P*<0.0001, SS=Sum of squares, df=Degree of freedom, MS=Mean of squares, F=One way ANOVA test

of various pesticides during spraying season, a cumulative effect of pesticides bring about depression in activity of SChE. Out of 183 sprayers, 126 were exposed to a single pesticide (58, OP; 40, neonicotinoid; 28, miscellaneous e.g., topic, endosulfan, leader matador, etc.), whereas, 44 sprayers used combination of two pesticides and six sprayers were exposed to a mixture of three pesticides as shown in Table 7. The maximum depression in SBChE was observed in the sprayers exposed to a single pesticide as most of them used different types of OPs. Similarly, among the sprayers using mixture of pesticides, statistically more significant depression in SBChE was seen in either the sprayers using mixture of OP pesticides or combination of OP and organochlorine pesticides.

In this study, the pesticides used by sprayers showed significant decrease in S ChE values in the following orders: Round up > Rift > Monocrotophos > DDVP > Chlorpyrifos > Profenofos > Confidor. Also, the toxicity of the pesticides has been related to their ability to inhibit acetylcholinesterase.[12] The pesticides Malathion, Dizinon, Cholpyrifos, and Dichlorovos were found to be strong inhibitors of BuChE.[13] Likewise, inhibition of AChE by OP pesticides was recorded in the order of Chlorpyrifos > Monocrotophos > Profenofos > Acephate.[14] Moreover, Chlorpyrifos and Malathion are observed to be preferential inhibitors of BChE, while Dimethoate predominantly inhibits AChE.[15] Organophosphorus are the most dangerous among all synthetic compounds. Chemically, they contain an organophosphorus radical, amino radical ($-NH_2$), and phenyl radical. Carbamates are less toxic than OPs, because, they are rapidly hydrolyzed to methyl ceramic acid and variety of phenol substances which are of low-toxicity.[16] Thus, these results clearly indicate that the sensitivity of the biomarkers AChE and SChE can be assessed according to the chemical nature of the pesticide and its mode of action.

Human exposure to cholinesterase inhibiting pesticides can result from inhalation, ingestion, eye or skin contact during manufacture, mixing or applications of these chemicals. Anyone exposed to cholinesterase inhibiting pesticides can develop lowered cholinesterase levels. The sprayers used mainly the retail shop owner as the information source for knowledge regarding the pesticide they used. Some also consulted other farmers and none of the farmers consulted government or any other agricultural authorities as their source of information. Thus, it became tedious for us to identify some pesticides e.g., Leader, Topic, etc.

In normal transmission of a nervous impulse from nerve to nerve, acetylcholine is released into synapse to excite the receiving neurons. Unless acetylcholine is rapidly broken-down, the receiving nerve is constantly fired resulting in uncoordinated muscle movement, nausea, dizziness, and eventually seizures and unconsciousness. The enzyme acetylcholinesterase is responsible for the expedient breakdown of the neurotransmitter acetylcholine. Thus, cholinesterase is one of the most important enzymes required for the proper function of nervous system. Nowadays, OPs and carbamates are the predominant types of pesticides used and are found to be highly toxic to humans and other organisms.[17] Organophosphorus pesticides inhibit cholinesterase irreversibly and hence promote the accumulation of acetylcholine at nerve synapses. The excessive accumulation of acetylcholine in the synapses induces hyperactivity in cholinergic pathway. Many workers have correlated cholinesterase inhibition with adverse health effects and may induce symptoms ranging from increased salivation and headache to convulsion and suppressed breathing which can have a fatal outcome.[18]

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Table 7: Comparison of cholinesterase according to combination of pesticides sprayed by spray workers with control

	Classification of pesticides	Pesticides used	Number of individuals (*n*)	Level of cholinesterase	*t* value
Controls			100	7551.71	
Sprayers who used single pesticides (*n*=126)	Organophosphate, Neonicotinoid, Organochlorine, Phosphanoglycine, Pyrethorid, Novaluron, and Carbamate	Profenofos (OP); Monocrotofos (OP); DDVP (OP); Acephate (OP); Acetamiprid (neo); Confidor (neo); Endosulfan (OC); Rift (OC); Matador (PTD); Leader; Monochloroacetic acid; Actara; Butachlor	133	5301.01	8.39***
Sprayers who used combination of two pesticides (*n*=44)	Neonicotinoid and Organophosphate	Confidor (neo) and Profenofos (OP); Confidor (neo) and Monocrotofos (OP); Confidor (neo) and Acephate (OP); Confidor (neo) and DDVP (OP); Acetamiprid (neo) and Chlorpyrifos (OP); Acetamiprid (neo), and Acephate (OP)	28	6454.28	3.76***
	Neonicotinoid and Neonicotinoid	Confidor (neo) and Acetamiprid (neo);	07	6611.71	
	Organophosphate and Organochlorine	Monocrotofos (OP) and Endosulfan (OC); Monocrotofos (OP), and Rift (OC)	03	5652.67	
	Organophosphate and Organophosphate	Monocrotofos (OP) and DDVP (OP)	01	3387	
	Neonicotinoid and Pyrethorid	Confidor (neo) and α-cypermethrin (PTD)	01	6503	
	Neonicotinoid and Novaluron	Confidor (neo) and Rimon (NoN)	01	7087	
	Organophosphate and Carbamate	Chlorpyrifos (OP) and Dithan M-45	01	4873	
	Neonicotinoid and Topic	Confidor (neo) and Topic	01	4218	
	Organophosphate and Pyrethroid	Acephate (OP) and Matador (PTD)	01	5151	
Sprayers who used combination of three pesticides (*n*=06)	Neonicotinoid and Organophosphate	Monocrotofos (OP), Profenofos (OP), and Confidor (neo)	01	8680	1.68*
	Organophosphate, Organochlorine, and Neonicotinoid	Monocrotofos (OP), Endosulfan (OC), and Confidor (neo)	01	6590	
	Organophosphate, Pyrethroid, and Neonicotinoid	Monocrotofos (OP), Matador (PTD), and Confidor (neo)	01	6837	
	Organophosphate, Organochlorine, and Phosphanogycine	Monocrotofos (OP), Rift (OC), and Roundup	01	2874	
	Organophosphate, Organophosphate, and Pyrethorid	Monocrotofos (OP), DDVP (OP), and α-cypermethrin (PTD)	01	3287	
	Neonicotinoid, Organophosphate, and Neonicotinoid	Confidor (neo), Acephate (OP), and Acetamiprid (neo)	01	7220	

***Significant at *P*<0.0001, *Significant at *P*<0.05, OP=Organophosphate, neo=Neonicotinoid, OC=Organochlorine, PTD=Pyrethorid, CM=Carbamate, DDVP=Dichlorovinyl dimethyl phosphate

In the present study, abnormal SChE levels were observed in sprayers having more exposure period (>5 years) as well as in those workers in which SChE was assessed after 28 days of spray. A positive correlation was found between SChE activity and years of exposure and is also confirmed by ANOVA. The faster recovery in SChE concentration may reflect an adaptive response to long term exposure to pesticides, i.e., it might leads to a higher SChE induction that would reduce OP binding to biological targets.[19] It may be due to some genetic mutation or to quick breakdown of pesticides based on higher tolerance or probable resistance.[20] It is also believed that these workers had more experience and were more careful while handling pesticides.[21]

A significant decrease in SChE activity ($P < 0.0001$) was observed in the age group less than 25 years of age. This could be explained partly by the fact that younger farm workers tend to be engaged in more risky activities. In this study, most of the young sprayers showed decline in SChE concentration in comparison to controls. The occupational sprayers who sprayed pesticides more than three times per month were more likely to develop abnormal SChE levels than seasonal workers or day-laborers; this may be due to the fact that permanent farm workers had longer and more frequent direct exposure to pesticides.[22] Moreover, longer exposures without correct use of personal protective equipments (PPE) resulted in higher risk of developing abnormal SChE levels and health hazards.[23] It is also suggested that younger seasonal farm workers or day laborers might be given tasks which bear greater risk of exposure to pesticides.[24]

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In this study, BMI also showed a positive co-relation with SChE inhibition. Similar views were also expressed by other researchers.[21,25] It can be correlated to the liver size, where SChE is synthesized before being delivered to the blood or with fat deposition in liver tissue which would stimulate the hepatic synthesis of PChE.[26]

In this study, we compared the SBChE levels between exposed and unexposed subjects. However, to detect such a decrease in risk subjects, a baseline activity measurement prior to exposure and follow up measurements are necessary. But this is not always possible in practice.

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