

Chemical Biomarker Study of Negative Physiological Effects of Insecticides on Pakistani Farm Workers Health in the Central Punjab

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Abstract

Background: In recent decades, use of pesticides in agricultural practices has posed threat to human population.

Objectives: Present research was designed to explore the adverse effects of insecticides on 339 individuals (study group farmers $n = 256$ and control $n = 83$) selected from 62 different sites of central Punjab. The sample was matched on socio-economic status and age.

Methodology: A structured questionnaire was used to

obtain the demographics and pesticide related details. For bio-chemical analysis blood samples were collected from both groups.

Results: Bio-chemical analysis shows that, farmers exposed with pesticide have significant elevated levels of Urea ($p = 0.05$) and alanine transaminase (ALT) ($p = 0.01$) as compared with control. On contrary, low levels of BChE, albumin and total protein (TP) levels ($p = 0.001$; $p = 0.05$ and $p = 0.001$ respectively) were found in pesticide exposed subjects than controls. Also, significant increase in serum creatinine ($p = 0.01$), aspartate transaminase (AST) ($p = 0.01$) and notable decrease in the albumin, TP and BChE ($p = 0.001$) were evident in farmers with poor protective measures. However the statistical difference is not correlated with clinical difference.

Conclusions: Several biomarkers have shown the possible hazards of pesticides to farmer's health than controls. It was further explored that health indicators also associated with other factors like duration of exposure to pesticides, tobacco smoking and poor handling practices amongst the studied population.

Key words: Pesticides, Biomarkers, Environmental toxicology, Public health, Butyrylcholinesterase (BChE) activity, LFT, RFT, practices.

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tional worth of food as well.^{2,3} In Pakistan, agricultural products contribute 21% of the total Gross Primary Productivity (GDP) and pesticide use is on increase as from last 20 years, its usage and number of sprays per crop has been increased by 11.9% and 10% respectively.⁴ According to the 'Economic Survey (2006), alarming situation has developed for the agrarian workers because pesticides usage has risen during 1980 to 1999 from 665 tons to 45,680 tons respectively. At present, a variety pesticide like insecticides, 108 different types; weedicides, 39 types; fungicides, 30 types; agricides, 5 types, and rodenticides, 6 types, are being in use in Pakistan.⁵ It has been reported by many researchers that particulars of the human health impacts depends on pesticide exposure which may be accidental,⁶⁻⁸ or occupational.⁹⁻¹¹

Although pesticides are playing significant role in enhancing crop production to meet the demand of ever – increasing human population but at the same time very harmful to the non-target biota in the vicinity of pesticide use including industrial and agricultural workers.

In the developing countries, exposure to pesticides is considered as a major occupational risk among farmers.¹²⁻¹⁴ The exposure to pesticides may occur due to spillage, improper usage, or non-caring attitude of pesticide handlers. Miss handling of pesticides while transfers may also cause human exposure to pesticides.¹⁵ For determining health risks during farming practices in underdeveloped countries, dermal exposure to pesticides is of particular interest and it is primarily due to the lack of work – related hygiene policies.¹⁶

Farm workers that have poor information regarding safety practices and protective measures are more at risk to pesticide exposure.¹⁷ Chronic low dose exposure to pesticides causes health effects and is very difficult to evaluate as these chemicals are mostly used in the form of a variety of mixtures.^{18,19}

The pesticide residues tend to accumulate in various tissues and subsequently could affect the metabolic system of the body including enzymes.^{20,21} However, some recent studies suggested strong correlation between pesticide exposure and rise in the values of various liver and kidney related enzymes and other factors, such as alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphate (ALP), total protein (TP), urea, creatinine, bilirubin, total proteins etc.²²⁻²⁴ Kidney and liver functions may be affected due to extended exposure to variety of pesticide. A significant decrease in butyrylcholinesterase (BChE) has been previously observed in humans exposed to

pesticides compared to those not exposed.²⁵

Different parameters, such as attitude, knowledge and practice about handling of pesticides have direct bearing on pesticide exposure. This necessitates initiation of such studies, particularly in the under developed countries, like Pakistan.

Patients and Methods

The present study was designed to investigate the fatal effects of commonly used insecticides on the Pakistani farm workers' health in the area of central Punjab. This is basically a chemical biomarker study based on human health concerns. This study has followed ex-post facto research design with comparison group. In ex-post facto research, researcher compares pre-existing groups (exposed and control) on dependent variables. One group was exposed group comprises of farmers having acquaintance of pesticide spray. The second group comprises of individuals living in same vicinity but not at all exposure to pesticide spray. The study involved the following:

Sample and sampling.

Selection of individual farm workers exposed to insecticides (exposed group).

Selection of matched control (individual with no exposure of pesticide, living in same vicinity as the exposed sample).

Collection of blood samples from the experimental group.

Determination of biochemical parameters of collected blood samples including serum isolation, renal and hepatic enzyme functions, Butyrylcholinesterase (BChE) determination.

Analysis of blood sample results.

The first and important step of study was to select the sites which are exposed to continuous spray of insecticides. A rational was made on the basis of literature that in vegetable (crops like cabbage, brinjal, pea, potatoes) growing areas, more spray needed than other crops. These sites exists in the region of Central Punjab (Lahore, Kasur, Sheikhpura and Nankana Sahib, Fig. 2), Pakistan (Fig. 1). A total of 339 individuals (pesticide exposed farmers $n = 256$ and control $n = 83$) were selected for the study. Fourteen participants withdrew from the research as per their own preference and hence not counted in total sample size. It was purposive sampling.

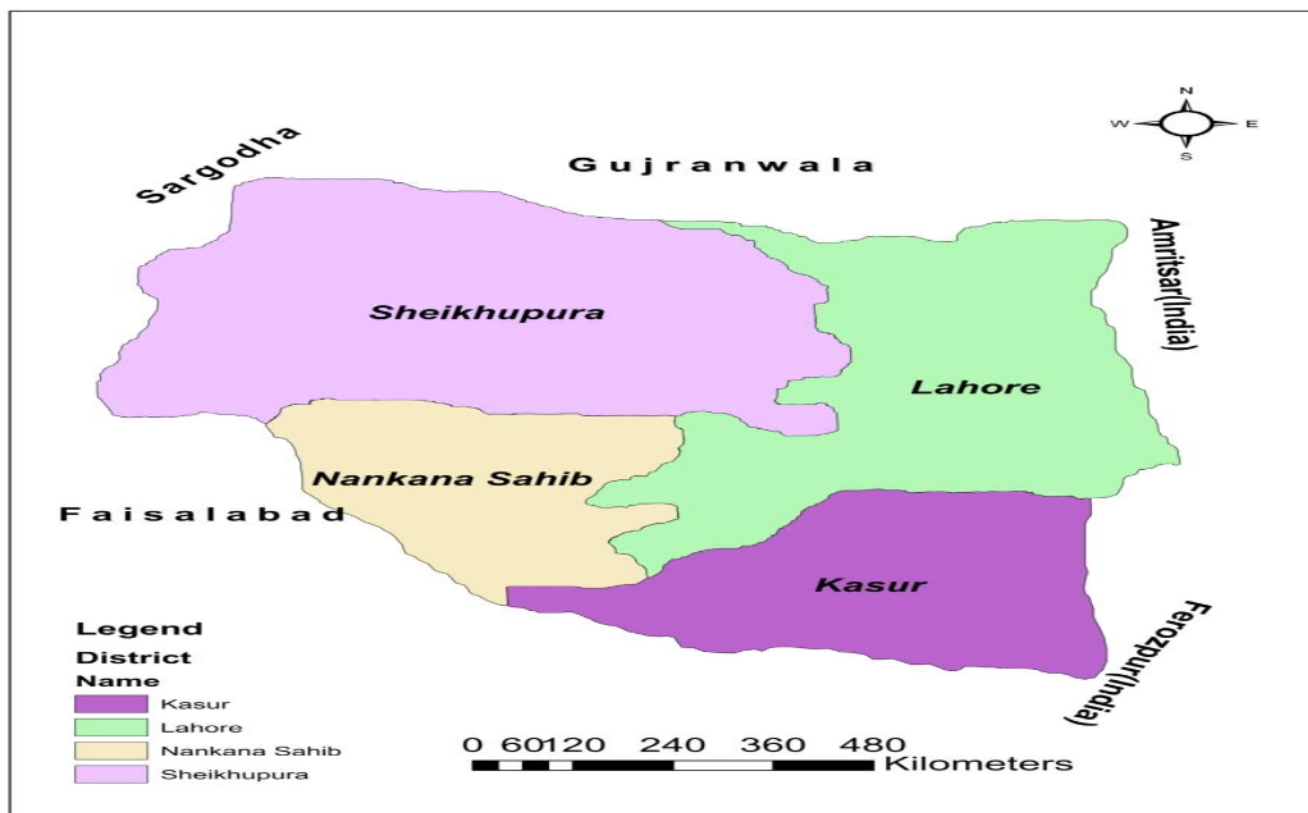


Figure 1: Map of the study area showing sampling sites of Kasur, Lahore, Nankana Sahib and Sheikhupura in Punjab Province, Pakistan (source: google maps).

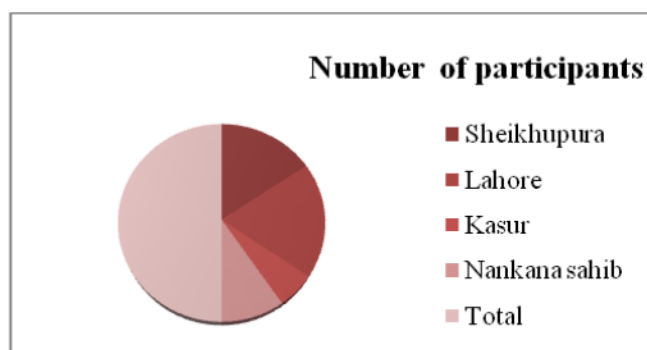


Figure 2: Pie chart showing proportion of participants from different districts of Punjab, Pakistan.

Inclusion criteria for the farmers was continuous exposure of insecticides spray such as chlorpyrifos (an organophosphate insecticide) and/or imidacloprid (a neonicotinoid insecticide). They are using these insecticides extensively in the agricultural fields by at least from the last five years. On the other hand, those individual were selected as sample for control/comparison group who were not involved in spray of pesticides.

The sample groups were matched on age and socio-economic status. It was ensured that all subjects with apparent disease were excluded from research.

The study span stretched from October 2013 to September 2014.

First permission was taken from the Departmental Board of Studies and Advanced Studies and Research Board of GC University Lahore. A signed consent letter was taken from each individual participating in this study. They were told about the aims and objectives of the study and their role. They were explained that their blood sample (through syringe of 5 ml) will be taken, by using high standard procedure for scientific research, by a trained male nurse. They were further told that their participation in the study will be voluntary and they are participating in the research on their will and could withdraw from research if they want, at any instance during the study. Only those participants were included who gave written consent. Only fourteen participants (nine from study exposed group and five from control group) dropped – out during the study and were not included in the total head count.

3 ml of blood samples from all study individuals

were collected through venipuncture into a gel – coated tube. Each tube was assigned an identification number, with sampling date, time, area and demographics of the sampled individuals (Figure 3 – 4).

Blood serum was collected from the samples tubes which were without any anticoagulant. The blood samples were allowed to stand for few hours. The samples were then centrifuged at 4000 rpm for 5 – 7 minutes and serum was isolated. The serum was pipette out and stored at -20°C for subsequent analyses.

For all the blood samples, same measurement Criteria was used; blood serum was analyzed to determine different biochemical parameters, such as urea, alanine transaminase (ALT), alkaline phosphate (ALP), serum creatinine, bilirubin, aspartate transaminase (AST) serum albumin, and total protein (TP) by following instructions of diagnostic kits using fully automatic chemistry analyzer (AU 480; *Beckman Coulter*). This study was performed at the certified pathological laboratory of Jinnah Hospital, Lahore, Pakistan.

BChE was determined by colorimetric method following the kit instructions of Randox (Cat No. CE190) using the semiautomatic analyzers (Metro Lab. 1600 DR) in a certified pathological laboratory of District headquarter hospital of Sheikhpura (DHQ – SKP), Punjab, Pakistan. Moreover all blood samples were preceded through same criteria for the measurement of BChE.

Collected data were statistically analyzed using Statistical Program for Social Sciences (SPSS, version 18). The frequencies, descriptive analysis, independent sample *t*-test, analysis of variance (ANOVA) were performed. Results were considered significant if *p*-values were ≤ 0.05 .

Results

No result was dropped out and Most of the farmers were found with poor protective measures. Only 5.5% of the farmers used long shoes / boots and special spectacle during the spraying activity. Moreover, 92.5% of farmers had never been used the special safety trouser. Only few farmers used cap/turban (6.6%), gloves (5.1%) or mouth mask (5.1%) during the spray. Similarly, washing of hands, changing of cloths after spray and wear of gloves during the solution preparation was observed in 5.5%, 5.1% and 4.7% farmers, respectively. Very few farmers were aware of the preparation of pesticide dose (5.5%), knowledge of mixing of pesticides (5.5%), loading of pesticide in machine (5.9%) and knowledge of maintenance of spray machine and other related equipment (5.7%).

The Pesticides exposed farmers/spray operators had significantly higher levels of urea and ALT as compared to the control subjects moreover the levels of BChE (an already established marker for pesticide exposure) decreased significantly in farmers as compared to their respective controls. Similar trends were observed for albumin and total proteins (Table 1).

A significant increase in the levels of serum creatinine ($P = 0.01$) and AST ($P = 0.01$) among pesticides – exposed farmers/spray operators was recorded in the subjects who had never used the protective measures was observed in comparison to the subjects who occasionally, frequently, or always used the protective measures. However, there was notable decrease in the albumin, total proteins, and BChE ($P = 0.001$) in the subjects who had never used the protective measures (Table 2).



Figure 3 – 4: Interviews and physical examination of male farm workers and collection of Blood sample from a sprayer (all of farm workers are without any protective measures e.g. hand gloves, face, eyes, nose or mouth mask).

Table 1: Comparisons of biochemical parameters of pesticide exposed subjects (farmers) with pesticide non-exposed subjects (Independent *t*-test).

	Farmers Versus Control				
Biochemical Parameter's	NW	M	SD	t	P
Urea (MG/DL)	Farmer	30.64	13.89	2.062	0.05
	Control	27.23	9.97		
ALT (SGPT; U/L)	Farmer	26.25	20.43	2.435	0.01
	Control	22.15	16.90		
ALK. PHOSPHATE (U/L)	Farmer	92.69	51.47	1.812	0.072
	Control	96.28	39.05		
S/Creatinine (MG/DL)	Farmer	0.76	0.21	-.668	0.505
	Control	0.75	0.22		
Bilirubin T (MG / DL)	Farmer	0.51	0.24	.284	.0777
	Control	0.50	0.20		
AST (SGOT; U/L)	Farmer	40.89	27.81	.117	.0907
	Control	33.90	16.93		
ALB (G/DL)	Farmer	3.74	0.72	2.742	0.05
	Control	4.39	0.38		
TP (G/DL)	Farmer	7.21	1.39	-10.55	0.001
	Control	8.60	1.08		
BChE (U/L)	Farmer	4432.11	946.25	-25.83	0.001
	Control	8025.69	1471.72		
n =339, NW (nature of work); farmer = 256, control = 83					

In Farm workers (having poor pesticide handling practices) albumin and total proteins levels declined significantly ($p = 0.001$) (Table 3).

Majority of the studied subjects had dealt with pesticides for at least 5 years. In the farmers/spray operators, ALT, serum creatinine and AST levels were significantly higher ($p = 0.01$ and $p = 0.001$, respectively) but the serum albumin, total protein and BChE declined significantly ($p = 0.001$) in the subjects with higher duration of pesticide exposure (Table 4).

It was also noted that most of the farm workers (73.8%) were smokers. In farmers/spray operators, due to exposure to pesticides, urea (smokers, mean = 31.83 ± 15.52 mg/dl versus non-smokers, mean: 27.29 ± 6.51 , $p = 0.05$), ALT (smokers, mean: 28.86 ± 22.28 u/l versus non-smokers, mean: 21.24 ± 17.09 , $p = 0.01$), serum creatinine (smokers, mean: 43.18 ± 29.87 mg/dl versus non-smokers, mean: 34.44 ± 19.72 mg/dl, $p = 0.05$) and AST (smokers, mean: 31.83 ± 15.52 u/l versus non-smokers, mean: 27.29 ± 6.51 u/l, $p = 0.05$) lev-

els increased significantly in smokers than in non-smokers; however, serum albumin (smokers, mean: 3.69 ± 0.76 g/dl versus non-smokers, mean: 3.88 ± 0.58 g/dl, $p = 0.05$), total protein (smokers, mean: 7.06 ± 1.41 g/dl versus non-smokers, mean: 7.54 ± 1.23 g/dl, $p = 0.01$) and BChE (smokers, mean: 4106.80 ± 851.85 u/l versus non-smokers, mean: 5349.80 ± 484.14 u/l, $p = 0.001$) levels decreased (Table 5).

Discussion

Hazardous impacts on the health of the human population under investigation and exposed to pesticides, such as organophosphates, neonicotinoids, pyrethroids, carbamates and organochlorines etc. have been found. Various parameters have been discussed as follows.

Pesticide exposure in farmers seems to stimulate significant rise of urea and alanine transaminase

Table 2: Comparison of biochemical parameters in relation to the protective measures use habits by the farm workers (one way ANOVA).

Biochemical Parameters	Protective Measures	N	M ± SD		F	P
S/ Creatinine (MG/DL)	Never	18	0.88	± 0.22	3.44	0.01
	Occasional	27	0.82	± 0.21		
	Frequent	117	0.75	± 0.21		
	Always	94	0.73	± 0.21		
AST (SGOT; U/L)	Never	18	57.5	± 39.75	3.67	0.01
	Occasional	27	48.54	± 41.29		
	Frequent	117	39.82	± 25.46		
	Always	94	36.84	± 21.39		
Albumin (G/DL)	Never	18	2.93	± 0.85	16.78	0.001
	Occasional	27	3.36	± 0.85		
	Frequent	117	3.74	± 0.71		
	Always	94	4.01	± 0.48		
Total Protein (G/DL)	Never	18	5.70	± 1.90	13.86	0.001
	Occasional	27	1.70	± 1.08		
	Frequent	117	7.13	± 1.40		
	Always	94	7.68	± 1.02		
BChE (U/L)	Never	18	2763.60	± 448.24	714.49	0.001
	Occasional	27	3318.90	± 566.11		
	Frequent	117	4088.80	± 285.68		
	Always	94	5498.70	± 173.65		

Significance confidence, $p \leq 0.05$ and 0.01

Table 3: Analysis of the effect of pesticide handling expertise on the biochemical parameters of farm workers exposed to pesticides (one way ANOVA).

Biochemical Parameters	Pesticides Usage Expertise's	M	SD	F	P
Albumin (G/DL)	Very poor	3.59	0.78	4.12	0.001
	Poor	3.85	0.71		
	Satisfactory	3.94	0.49		
	Good	4.01	0.49		
TP (G/DL)	Very poor	6.86	1.48	5.74	0.001
	Poor	7.45	1.29		
	Satisfactory	7.68	0.96		
	Good	7.60	1.14		

N = 256; Pesticides usage expertise; very poor, 132, poor, 72, satisfactory, 38, good, 14) Significance confidence, $p \leq 0.05$ and 0.01

Table 4: Study of biochemical parameters in farm workers in relation to exposure duration to pesticides (One way ANOVA).

Biochemical Parameters	Exposure Duration	Farmer	F	p
		M \pm SD		
Urea (MG/DL)	15 years or more****	32.45 \pm 11.31	2.172	.092
	10 years or more***	34.78 \pm 18.55		
	5 years or more**	31.23 \pm 14.98		
	1 years or < 5 years *	28.2 \pm 9.97		
ALT (SGPT; U/ L)	15 years or more****	39.18 \pm 30.52	3.385	0.01
	10 years or more***	33.23 \pm 28.17		
	5 years or more**	26.9 \pm 18.55		
	1 years or < 5 years *	23.05 \pm 19.42		
	Total	26.86 \pm 21.28		
Alk. Phosphate (U/ L)	15 years or more****	89.82 \pm 32.62	1.428	.235
	10 years or more***	109.35 \pm 84.76		
	5 years or more**	89.69 \pm 47.66		
	1 years or < 5 years *	90.51 \pm 40		
	Total	92.69 \pm 51.47		
S/Creatinine (MG/DL)	15 years or more****	0.92 \pm 0.23	3.83	0.01
	10 years or more***	0.83 \pm 0.2		
	5 years or more**	0.75 \pm 0.21		
	1 years or < 5 years *	0.73 \pm 0.21		
	Total	0.76 \pm 0.21		
Bilirubin T (MG/DL)	15 years or more****	0.46 \pm 0.21	1.496	.216
	10 years or more***	0.57 \pm 0.21		
	5 years or more**	0.51 \pm 0.25		
	1 years or < 5 years *	0.48 \pm 0.22		
	Total	0.51 \pm 0.24		
AST (SGOT; U/L)	15 years or more****	63.45 \pm 47.74	5.132	0.001
	10 years or more***	51.16 \pm 40.55		
	5 years or more**	39.11 \pm 23.74		
	1 years or < 5 years *	36.66 \pm 21.35		
	Total	40.89 \pm 27.81		
Serum Albumin (G/DL)	15 years or more****	2.79 \pm 0.85	16.539	0.001
	10 years or more***	3.34 \pm 0.84		
	5 years or more**	3.73 \pm 0.72		
	1 years or < 5 years *	4 \pm 0.48		

	Total	3.74 ± 0.72		
Total Protein (G/DL)	15 years or more****	4.66 ± 1.19	21.216	0.001
	10 years or more***	6.84 ± 1.21		
	5 years or more**	7.12 ± 1.41		
	1 years or < 5 years *	7.68 ± 1.02		
	Total	7.19 ± 1.38		
BChE (U/L)	15 years or more****	2465.91 ± 301.51	728.50	0.001
	10 years or more***	3394.94 ± 745.52		
	5 years or more**	4054.47 ± 93.38		
	1 years or < 5 years *	5499.04 ± 172.75		
	Total	4432.11 ± 946.25		

(Farmers; Total n =256, **** = 11, *** = 35, ** =115, * = 5 Significance confidence, $p \leq 0.05$ and 0.01
 NW = nature of work, ALT (SGPT) = alanine transaminase, AST (SGOT) = aspartate transaminase, ALB = Serum Albumin, Bilirubin T = Bilirubin Total, S/Creatinine = Serum Creatinine, TP = Total Protein, BChE = Butyrylcholinesterase

Table 5: Comparison of biochemical changes between the Smokers and non-smoker groups of farm workers.

Biochemical Parameters	Smoking Habits	M ± SD	F	P
Urea (MG/DL)	Smokers	31.83 ± 15.52	5.371	0.05
	Non-smokers	27.29 ± 6.51		
ALT (SGPT;U/ L)	Smokers	28.86 ± 22.28	6.473	0.01
	Non-smokers	21.24 ± 17.09		
S/ Creatinine (MG/DL)	Smokers	0.78 ± 0.21	3.322	0.05
	Non-smokers	0.72 ± 0.21		
AST (SGOT;U/L)	Smokers	43.18 ± 29.87	4.963	0.05
	Non-smokers	34.44 ± 19.72		
Serum Albumin (G/DL)	Smokers	3.69 ± 0.76	3.346	0.05
	Non-smokers	3.88 ± 0.58		
Total Protein (G/DL)	Smokers	7.06 ± 1.41	5.951	0.01
	Non-smokers	7.54 ± 1.23		
BChE (U/L)	Smokers	4106.80 ± 851.85	127.81	0.001
	Non-smokers	5349.80 ± 484.14		

N = 256; smokers = 189; Non-smokers = 67; Significance confidence, $p \leq 0.05$ and 0.01

(ALT), whereas there was a tendency of decline in the levels of albumin and total proteins in the pesticides – exposed population (farmers) compared to non-exposed subjects (Table 1). These findings are in agreement

with a number of previously reported similar studies.²⁶⁻³⁰ A recent study, using mice as a model animal, revealed that deltamethrin (a synthetic pyrethroid-pesticide) caused decrease in total proteins.³¹ The dec-

rease of serum proteins, especially albumin, might be due to alteration in protein metabolism of free amino acids and their production in liver under the influence of pesticides.³² Another explanation of protein decline may be the result of protein loss either due to protein synthesis reduction/degradation or increase in the proteolytic activity.³³ Protein metabolism results in the production of urea and creatinine as waste products that are needed to be excreted through the kidney. The marked increase in levels of serum urea and creatinine are indicators of kidney function impairment.³⁴ The potential increase in the levels of ALT indicated the damages to the liver.³⁵ This implies that the pesticides used in the study are organophosphates; neonicotinoids, pyrethroids, carbamates and organochlorines etc have exerted suppressive impact on protein anabolism as well as resulted in the deteriorating functions of liver and kidney.

Butyrylcholinesterase (BChE) is a non-specific enzyme that can cause hydrolyses of a lot of diverse choline esters. BChE is reliable biomarker and confirmed indicator of organophosphate exposure.³⁶ In humans, primarily it is present in the liver.³⁷ and is determined by the BCHE gene.³⁸ The decline in BChE level can cause the delay in the metabolism of some of clinically significant compounds, such as succinylcholine, procaine, mivacurium, cocaine and heroin, etc.³⁹

Depressed level of butyrylcholinesterase (BChE) levels in spray operators/farmers in comparison to their respective controls in this study confirms the adverse impact of exposure of organophosphates to these populations. This theory is further strengthened by the findings significant variations in the values those using protective measures as compared to those not using any of these. Similar findings have already been reported in other related studies previously conducted on farmers handling pesticides.⁴⁰⁻⁴⁴ For instance, in a pesticide – exposed population in India, significant decrease in cholinesterase level was reported.⁴⁵ Several other studies have also suggested a decrease in BChE level in response to exposure to organophosphates.^{46,47} Similarly, in other parts of the developing world, including Mexico, Kenya, and Bangladesh, similar findings have been reported.^{45,48,49} Varying responses of the populations exposed to insecticides by varying degree indicate that the impact rendered on part of these insecticides depends on a number of factors, such as the duration of exposure, the use of protective measures, etc.

One of the major causes of this scenario is that

there is no strict implementation of “personal protection guidelines” by the local government or any other relevant authority in Punjab. These observations reveal that most of the farmers never use any protection (Figure 3 – 4).

Among the pesticide-exposed farmers/spray operators, a significant increase in serum creatinine and AST but on the other hand, significant decrease in albumin, total proteins, and BChE (Table 2) again show even worse situation of health profile of this population. This implies that the risk of pesticide toxicity is directly proportional to the extent of exposure. No or inappropriate use of protective measure involved in his business lead to more influx of the insecticides in the body, either through inhalation, skin contact or even through exposed living surface, such as eyes, etc.; this might lead to altered physiological and biochemical processes.

Similar findings have been suggested by the previous studies concerning insecticide toxicity.⁵⁰

Similar trend was also reported by a study carried out in Egypt where significant incidence of health related problems were reported in agricultural community.^{51,52,53}

Another study also pointed out that the incidence of pesticide exposure increased among workers who had not followed instructions / guidelines for proper handling of pesticides.^{15,54,3}

Significantly altered biochemical responses in the populations under investigation with more number of years (< 10) and particularly that BChE levels (Table 4) support the idea that the toxicity of insecticide increases with the passage of time. These findings are in agreement with the previously undertaken study on agricultural workers in Kenya, where cholinesterase inhibition was recorded due to prolonged exposure of the workers to pesticides.⁴⁹ Similarly the elevated levels of ALT in more exposed population in our study endorse the previously reported similar findings.⁵⁵

Elevated levels of vital biochemical indicators, such as urea, ALT, serum creatinine, and AST and the depressed values of serum albumin, total proteins, and BChE levels (Table 5) in smokers (comprising of major proportion of the studied population; 84% of farmers). These findings are also confirmed by already reported assumptions.^{56,57,27,58,59} This implies that tobacco smoking stresses vital physiological mechanisms and puts the body on elevated risk of insecticide toxicity.

Conclusion

Several biomarkers have shown the possible hazards of pesticides to farmer's health than controls. However, Farmers with less exposure duration to pesticides, good pesticides handling practices and non-smoking habits do not suffered much than as compare to farmers with poor pesticide handling practices and greater exposure duration.

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