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INFLUENCE OF EXPOSURE TO PESTICIDES ON LIVER ENZYMES AND CHOLINESTERASE LEVELS IN MALE AGRICULTURE WORKERS

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ABSTRACT

A significant increase in pesticide use has increased concerns about potentially adverse effects on human health and the environment. The study aimed to explore the effects of exposure to pesticides on the liver functions and acetycholinesterase levels in serum (AChES) and red blood cells (AChER) of 100 male participating in agricultural work ranging in age between 20 and 60 year with mean age 37.11±9.3. One hundred males matched for age and socio economic status were recruited as a control group to compare levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilriubin, alkaline phosphatase (ALP), total protein, AChES and AChER. The results indicate that AST, ALT and ALP were significantly ($P<0.05$) increased in pesticide-exposed workers compared with control. There was also a highly significantly ($P<0.01$) decrease in AChER among male agriculture workers compared with controls. At 10 - 50 times of pesticides applications there was a significantly ($P<0.05$) decrease in AChER and increase in AST, ALT and ALP activity among exposed group. However, there was a negative correlation between AST, ALT, direct bilirubin, and AChES and age among control group and a positive correlation between ALT and AST and age among exposed group. According to the number of pesticide application, there was a positive correlation between AST, ALP, total and direct bilirubin and number of pesticide application. Agricultural villages in Egypt require more attention to decrease the percentage of literacy among the farmers and raise their health awareness.

Keywords: Cholinesterase, Pesticides exposure, Liver enzymes, Agriculture, Male

1. Introduction

Pesticides are ubiquitous in the environment and have significant economic, environmental and public health impact. Their usage has played a significant role in raising the yields of crops from agricultural land around the world. The population of Egypt was about 19 millions in 1947 and reached 57 millions in 1992. In 2003, the population has grown to about 70 millions with a growth rate of ca. 2.5% annually. At the same rate of growth, the population of Egypt after 15 year may exceed 100 millions (Mansour, 2004). Thus, the growth rate of the population in Egypt is a very serious problem, which might be a threat to the future of this country. Subsequently, the balance between population increase and production of enough food is therefore one of the most important and challenging problems facing Egypt today. So, the use of pesticides in agriculture was, and will remain for the foreseeing future, an essential component and a prerequisite for increasing productivity of the land.

World-wide sales of crop protection chemicals in 1989 were projected to be about $21 billion (Hunter, 1989). Since the mid 1960s, the quantity of pesticides used in Egypt and Africa has increased about five-fold. About 1 million tons have been injected into the Egyptian environment during the last 40 years (Amr, 1999). Exposure to pesticides may involve large segments of population, which include agriculture workers and their families, besides the general population who may be exposed through home application of pesticides or via residues on food (Quandt *et al*., 2004). A vast majority of the population in Egypt is engaged in agriculture and is therefore exposed to the pesticides used in agriculture (Amer *et al*., 2002; Abdel Rasoul *et al*., 2008).

Exposure to pesticides both occupationally and environmentally causes a range of human health problems. It is estimated that nearly 10,000 deaths annually to use of chemical pesticide worldwide, with about three-fourths of these occurring in developing countries (Horrigan *et al*., 2002). Exposure to pesticides results in acute and chronic health problems (Yassi *et al*., 2001). The potential risks to pesticides applicator or farm worker occupationally exposed pesticides are greater than the risks to someone in the general population exposed only to traces of pesticides in food and /or water (Amer *et al.,* 2002). Exposure to low level of pesticides is known to produce a variety of biochemical changes, some of which may be responsible for the adverse biological effects in humans (Elhalwagy *et al*., 2009; Ibrahim *et al*., 2011). Conversely, some biochemical alterations may not necessarily lead to clinically recognizable symptoms, although all the biochemical responses can be used as markers of exposure or effect.

The aminotransferases are the most frequently utilized and specific indicators of hepatocellular necrosis. These enzymes-aspartate aminotransferase (AST) and alanine amino transferase (ALT) catalyze the transfer of the á amino acids of aspartate and alanine respectively to the á keto group of ketoglutaric acid. Alkaline phosphatases (ALP) are a family of zinc metaloenzymes, with a serine at the active center; they release inorganic phosphate from various organic orthophosphates and are present in nearly all tissues (Thapa and Anuj Walia, 2007). The liver enzyme has broad substrate specificity, including a variety of pesticide oxidations. It has been reported to be significantly lower in humans occupationally exposed to pesticides as compared to control (Zeinalov and Gorkin, 1990). Many researchers tried to correlate various enzymes with the harmful effects of pesticides, especially in the case of ALT**,** AST, ALP, and Acetylcholinesterase (AChE) (Altuntas *et al*., 2002; Ahmed and Mohammad, 2005; Remor *et al*., 2009; Vrioni *et al*., 2011; Dias *et al*., 2013). However, very little work has been done on this aspect in Egypt (Amer *et al.,* 2002; Mansour, 2004; Abdel Rasoul *et al*., 2008). Therefore, analysis of blood samples of agriculture workers compared with control was done to find 1) the impact of pesticide exposure on liver enzymes and AChE levels among different age group, 2) number of pesticide application and its effect on liver functions and AChE activity, 3) Correlation between liver enzymes, AChE and age and number of pesticide application.

2. Materials and methods

2.1. Study design and population

This is a cross section comparative study. As pesticides have been linked with various chronic diseases, individuals presenting diabetes, neurological disorders, liver dysfunction, or any other chronic condition were excluded from the population studied in order to avoid any interference with the biochemical parameters measured. Prior to the study, all the individuals gave informed and written consent and completed a detailed questionnaire, covering standard demographic questions, habits (sports, food, drugs, tobacco, etc.), as well as occupational, medical and family history, duration of pesticides application, kind of pesticides and personal protective equipment (PPE) used.

The study used 100 male pesticides appliers (sprayers) ranging in age between 20 and 60 years (mean age: 37.11±9.3) were recruited to participate in the study based on their potential for exposure to pesticides. They were from small villages (El-Oula, n=50 and Banger No. 25, n=50) at Banger El-Soukar agriculture sector, Alexandria, Egypt. The control group comprised 100 healthy male ranging in age between 20 and 60 year (mean age: 35.97±9.6) from the same geographical area who had no history of exposure to chemicals or other potentially genotoxic substances. In order to avoid differences in environmental exposure to pesticide residues, they were from the same geographical setting than applicators, so that their socio-economic and nutrition status was very similar. Characteristics of exposed and non-exposed groups are summarized in Table 1.

2.2. Sample collection and preparation

After an overnight fasting period, two samples of venous blood were collected in tubes with clot activator and EDTA (1mg/ml), respectively. Written consent was obtained from the subjects who agreed to participate, and they were allowed to drop out whenever they wanted. The samples were kept at 4 °C in a box with ice and transported to the laboratory. They were also protected from light to avoid enzymatic changes. Serum, plasma and erythrocytes were separated by centrifugation at 2000 rpm for 15 min. Plasma and serum was stored at -20 ^{o}C for biochemical analyses. Erythrocytes pellets were suspended and washed twice in Normal sterile saline (NSS) and diluted with an equal volume of saline (Hernández et al., 2005). Erythrocyte aliquots were diluted up to 1:100 in distilled water before storing overnight at (-20 ^{o}C).

2.3. Biochemical assays

Serum enzymes parameters were measured using Perkin Elmer UV/VIS spectrometer Lambda EZ201.

2.3.1. Liver function tests

Liver function tests comprising serum ALT, AST, ALP, and serum bilirubin (total and direct) were assayed according to Tietz, 1990. Total protein in serum was quantified by the procedure of Lowry *et al*. (1951), using bovine serum albumin (BSA) standard.

2.3.2. Acetyl cholinesterase

Acetyl cholinesterase activity in serum (AChES) and erythrocyte (AChER) was estimated spectrophotometrically at 405 nm by the method of Ellman *et al*. (1961), using acetylthiocholine iodide as the substrate. At pH 7.7, the cholinesterase catalyses esters choline hydrolyses as propionylcholine, and it liberates sulfidryl group thiocholine. The thiocoline reacts with acid 5,5'-ditiobis-2-nitrobenzoic (DTNB) producing a yellow compound directly proportional to the enzyme activity which is measured by spectrometer at 405 nm. Unit of enzyme activity was expressed as micromoles acetylthiocholine hydrolysed/min/ml blood fraction.

2.4. Statistical methods

All data are represented in tables as mean ± standard error (mean ± SE). Statistical analysis was performed using the SPSS package system version 11 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Socio-demographic characteristics of male agriculture workers

Table 1 shows that about 40% of the workers in Banger No. 25 and El Oula villages were illiterate. University graduates represented only 7% of the studied agriculture workers. The mean time of pesticide exposures and age (in year) in all farm workers were 11±6.05 and 37.11±9.3 year, respectively. All studied populations were not use personal protective equipment (PPE). The use of a wide range of pesticides – mostly of “moderately hazardous” to “slightly hazardous” category- among our study farmers (Table 2). Food and Agriculture Organization (FAO) recommends that World Health Organization (WHO) Ib (Highly hazardous) pesticides should not be used in developing countries (PAN, 2001). It also suggests that class II (Moderately hazardous) pesticides be avoided. But the practice of spraying these “powerful” pesticides continues. Preliminary results of environmental sampling tests done in the study area support this statement (Hassanin, 2009). Individuals are frequently exposed to many different pesticides or mixtures of pesticides, either simultaneously or serially, making it difficult to identify effects of particular agents. The relationship of pesticide-related cytotoxicity to overt clinical organ disease is still unresolved. In this regard, biomarkers may be used to detect the effects of pesticides before adverse clinical health effects occur.

Table 1: Characteristics of the study population

Characteristic	Controls	Exposed farm workers		
		Village		All farm workers
		El Oula	Bangar No.25	
No. of subjects	100	50	50	100
Age (in years) [mean±SD]	35.97±9.6	35.5±10.05	38.49±8.5	37.11±9.3
Years of exposure [mean±SD]	-	5±2.8	15±3.7	11±6.05
Personal protective equipment				
Yes	-			
No	-	50 (100%)	50 (100%)	100 (100%)
Education				
Illiterate	25	22	18	40%
Secondary school	33	24	29	53%
University graduate	42	4	3	7%

3.2. Pesticides exposure and liver functions among different age groups

Hepatotoxicity was monitored by quantitative analysis of the serum ALT, AST, ALP, bilirubin, AChES, and AChER and protein, which was used as the biochemical markers of liver damages. Liver enzymes (AST, ALT and ALP) were significantly (P<0.05) increased in pesticide-exposed workers compared with control among age group 20<60. There was a highly significantly (P<0.01) decreased in direct bilirubin and significantly (P<0.05) decreased in total protein compared with control. In the mean time there was no significant difference in total bilirubin content among exposed group as compared to control group (Table 3).

In age group 20<30 there was a highly significantly (P<0.01) increased and decreased in ALP and direct bilirubin, respectively, in exposed group compared with control. There was a significantly (P<0.05) increased in ALT, AST, and ALP and highly significantly (P<0.01) decreased in direct bilirubin in exposed group compared with control at the same age (30 <40). Among age group (40<50) we observed that AST was significantly (P<0.05) increased and direct bilirubin was highly significant (P<0.01) in exposed person compared with control. There was a highly significantly (P<0.01) increased in ALT in exposed group compared with control at age 50<60. Protein concentration was significantly (P<0.05) decreased in exposed group (40<50) compared with control at the same age (Table 3). An increased risk of liver dysfunction was observed in Air Force veterans responsible for the aerial spraying of herbicides in Vietnam, the effect being due primarily to increased AST, ALT, or LDH (Michalek *et al.,* 2001). ''In vitro'' studies have found that glyphosate and paraquat are able to inhibit certain enzyme activities: ALT, AST, lactate dehydrogenase (LDH), and acetyl cholinesterase (AChE) (El-Demerdash *et al*., 2001). Experimental studies in rats have reported significant changes in all these enzyme activities after subchronic administration of mancozeb in a dose-dependent manner (Kackar *et al*., 1999). Also, chronic exposure of rats and mice to OPs led to increased levels of serum ALT and AST (Gomes *et al*., 1999). Some pesticides, such as paraquat and glyphosate, have been reported to cause inhibition in the activity of serum AST and LDH, while other pesticides (OPs, organochlorines, and pyrethroids) are able to cause inhibition of LDH (El-Demerdash *et al*., 2001; Podprasart *et al*., 2007). Azmi *et al*., (2006) reported that a significant increase in the enzyme levels (ALT, AST and ALP) in different fruit and vegetable farm-station workers exposed to pesticides. The activities of serum transaminases may be raised due to increased release from non-liver tissue sources in various pathologies (Rej., 1989). Pesticide exposure causes leakage of cytosolic enzymes from hepatocytes and other body organs (Dewan *et al*., 2004). A high degree of abnormal liver function in agriculture workers may indicate toxic effects of pesticides and the presence of pesticides residues in blood. Altered liver enzyme activities have been reported among occupational workers exposed to organophosphorus pesticides alone or in combination with organochlorines (Amr, 1999). Ibrahim *et al*. (2011), reported that there was a significant elevation in serum liver enzymes (AST and ALT) in agriculture workers compared to the controls. Increased serum ALP activity may result from physiological or pathological enzyme production and release from non-liver tissue sources (Van Hoof and Broe, 1994). Fahimul-Haq *et al*. (2013) reported that the T. Bilirubin and D. Bilirubin in both groups were not only within the normal range but were also comparatively close to upper normal limit in pesticide industrial workers. High Bilirubin level after exposure to pesticides has also been reported by other researchers (Scharschmidt, 2000). It might be attributed to prolonged exposure to pesticides which disturbed the normal red blood cell metabolism, affecting the hepatic dysfunction and increased the level of bilirubin in the blood thereby causing hyperbilirubinemia which might be due to production of more bilirubin than the normal liver can excrete.

Table 2: Pesticides used by the exposed subjects

Pesticide	Common name	Chemical class	WHO
Insecticides	Chlorpyrifos	Organophosphorus compound	II
	Profenofos	Organophosphorus compound	II
	Glyphosate	Organophosphorus compound	III
	Penconazole	Organophosphorus compound	III
	Thiobencarb	Thiocarbamate	II

WHO (World Health Organization); II= moderately hazardous and III= slightly hazardous

3.3. Pesticides exposure and cholinesterase levels among different age groups

There was a highly significantly (P<0.01) decreased in the concentration of cholinesterase in red blood cell (AChER) of exposed groups (5155±1372) compared to control (6073±2688) in age group from 20 to 60 year. There was no significant difference in the level of cholinesterase in serum (AChES) of exposed group as compared to control within the same age group (Table 3). At the age groups 20<30 and 50<60 year, there were no significant differences in acetycholinestrase activity in AChER and AChES among exposed group as compared to control group. On the other hand there was a significantly (P<0.05) decreased in AChER and AChES activities in exposed age groups (30<40 and 40<50) compared with control (Table 3).

Results in the present study also referred that there was a highly significantly decreased in AChER among exposed group as compared to control group, while there was no significant difference in AChES in age group (20>60). Hazarika *et al*., (2003) reported that anilofos or malathion or their combination was significantly decreased AChER, plasma blood and brain as compared with control values. These finding are in agreement with the present study. According to Rawi, (1984), the pyrethroid decamethrin caused prolonged decrease in AChE activity in rats after single dose administration. Indeed, the main effects of pyrethroids are on sodium and chloride channels, as they modify the gating characteristics of voltage sensitive sodium channels to delay their closure (Bardberry *et al*., 2005). These agents increase Na^+ influx into synaptic terminals and create a hypopolarized and hyperirritable synaptic membrane, which in turn increases the release of the neurotransmitter acetylcholine (Rao and Rao, 1993).

3.4. Number of pesticide application and its effect on liver functions and acetylcholinesterase activity

Pesticide exposure can cause a variety of human health problems, both chronic and acute. Chronic effects are typically the result of low levels of exposure over a long period of time. These can occur even if there are no acute or immediate effects. Major health impacts from chronic exposure include cancers, reproductive and endocrine disruption, neurological damage, and immune system dysfunction (Sanborn *et al*., 2002). According to the WHO, long-term regular exposure to pesticides causes approximately 772,000 new cases of diseases every year (WHO and UNEP, 1990). There are very few studies on the long-term human health impacts of pesticides in Egypt. Many of the studies on pesticides in Egypt relate to pesticide residues in food, water and human bodies (Amer *et al*., 2002; Abdel-Halim *et al*., 2006; Sallam and Morshedy, 2008).

There was a significantly (P<0.05) increased in AST, ALT and ALP activity among exposed group as compared to control group at 10 - 50 times of pesticide applications. While, there was a highly significantly (P<0.01) decreased in total protein, and direct bilirubin content. At 50 - 100 times of pesticide applications there was no significant difference in AST activity, total bilirubine and protein content among exposed group as compared to control group (Table 4). However, there was a highly significantly (P<0.01) increased in ALT and ALP activity and highly significantly (P<0.01) decreased in direct bilirubine among exposed group compared with control group at the same conditions. As a result of increase the time of pesticide application (more than 100 times), there was no significant difference in AST, ALT, and total bilirubine among exposed group as compared to control group. While there was a significant decrease (P<0.05) in direct bilirubine and total protein content but there was a highly significantly (P<0.01) increased in ALP activity among exposed group as compared to control group (Table 4). High level of ALP was also reported by many researchers (Paulino *et al*., 1996; Mani et al., 2001; Altuntas *et al*., 2002) in the persons involved in pesticide spraying. High level of AST and ALT was also reported by other researchers in persons exposed to pesticides (Sahin *et al*., 2002; Rahman and Siddiqui, 2003; Azmi *et al.*, 2006).

At 10 - 50 times of pesticides applications there was a significant decrease (P<0.05) in acetylcholinestrase in red blood cell (AChER) among exposed group as compared with control group, while at 50 -100 and more than 100 time of pesticides applications there was no significant difference in AChER among exposed group. In the mean time at 10 -50, 50 -100 and more than 100 times of pesticides applications there was no significant difference in acetycholinestrase in serum (AChES) among exposed group as compared to control. The changes in cholinesterase levels were found to be significantly associated with pesticides exposure. The increase in individual cholinesterase levels was statistically significantly associated with environmental exposure to aerial pesticide application (Dalvie and London, 2006).

3.5. Correlation between liver functions, AChE and age and number of pesticide application

There was a negative correlation between AST, ALT, direct bilirubin, and AChES and age among control group and a positive correlation between ALT, and AST and age among exposed group. A positive correlation was found between total bilirubin and age in control and exposed group. At the same time there was a highly significantly (P<0.01) negative correlation between ALP and age among exposed group, while a positive correlation with age among control group. There was a positive correlation between AChER and age among control group and a negative correlation among exposed group. However there was a negative correlation between AChES, AChER and age in control and exposed (Table 5). According to the number of pesticide application, there was a positive correlation between AST, ALP, Total and direct bilirubin and number of pesticide application. On the other hand there was a negative correlation between ALT, total protein, AChES and AChER and number of pesticide application among exposed group (Table 6). Correlation between enzymes and pesticides has been reported by various workers, e.g., Misra *et al.*, (1985) who reported the high level of AST and ALT in the blood of occupational workers chronically exposed to organophosphate pesticides. Carvalho (1991), reported the increased level of AST and ALT in the persons due to occupational and environmental exposure of organochlorine insecticides in the state of Bahia, Naqvi and Khan (1993), correlated the inhibition of ALP by phosphine in Tribolium castaneum, which may be due to poisoning effect of Phosphine. Goel *et al.* (2000) also correlated a significant increase in the levels of various serum and liver marker enzymes such as ALP, AST and ALT due to the effect of chlorphyrifos. Rahman and Siddiqui, (2003), also showed the positive correlation between the enzyme activity (AST and ALT) in different tissues of rats exposed to phosphorothionate.

Table 3: Effects of chronic exposure to a mixture of pesticides on liver functions and acetylcholinestrase activity in male at different age

Parameters	Age groups (in year)														
	20 - 30			30 - 40			40 - 50			50 - 60			20 - 60		
	C (n=34)	Exp. (n=24)	*t(P)*	C (n=37)	Exp. (n=31)	*t(P)*	C (n=19)	Exp (n=39)	*t(P)*	C (n=10)	Exp (n=6)	*t(P)*	C (n=100)	Exp (n=100)	*t(P)*
ALT (U/l)	1.69±3.17	1.98±3.09	0.35 (0.72)	2.80±3.63	3.91±4.47	*2.10 (0.05)	1.37±4.05	7.34±15.84	1.60 (0.09)	0.90±1.79	7.12±8.01	2.40** (0.005)	1.98±8.01	4.97±10.62	2.6* (0.007)
AST (U/l)	8.96±10.14	7.59±9.98	-0.51 (0.581)	6.53±6.78	10.66±9.75	2.05* (0.045)	4.77±6.83	9.49±7.53	2.30* (0.025)	7.15±8.70	8.05±13.95	0.16 (0.34)	7.08±8.29	9.31±9.22	1.7* (0.027)
ALP (IU/l)	9.10± 4 .04	25.39±21.9 6	4.20** (0.001)	12.02±6.12	16.66±7.56	2.70* (0.031)	15.41±9.16	16.07±6.58	0.32 (0.132)	14.85±7.28	16.32±4.14	0.44 (0.43)	11.95±6.71	18.51±12.73	4.5* (0.050)
T. bilirubin (mg/dl)	0.45±0.34	0.45±0.18	0.042 (0.41)	0.55±0.38	0.60±0.23	0.63 (0.110)	0.52±0.39	0.56±0.26	0.00 (0)	0.65±.0.33	0.60±0.35	0.30 (0.7)	0.52±0.37	0.55±0.24	-0.68 (0.53)
D. bilirubin (mg/dl)	0.21±.0.16	0.03±.0.01	5.40** (0.000)	0.28±0.25	0.04±0.01	-5.30** (0.007)	0.23±0.21	0.04±0.02	-5.30** (0.008)	0.19±0.11	0.04±0.02	-2.90* (0.014)	0.24±0.20	0.04±0.01	9.9** (0.001)
T. protein (g/dl)	8.03±1.29	7.55 ±1.30	-1.30 (0.981)	8.91±1.47	8.21±4.72	-0.86 (0.27)	8.87±1.20	7.05±1.28	3.50* (0.007)	8.11±1.82	8.40±3.78	0.211 (0.199)	8.52±1.44	7.61±2.96	2.7* (0.024)
AChES (U/l)	4459.5±1739	5945±1480	1.40 (0.027)	7411±1203	4354±2266	7.10* (0.023)	5046±1703	3968±2303	1.80* (0.024)	3844.5±1818	3650±1115	-0.23 (0.818)	5601±7522	4543±3752	1.25 (0.2)
AChER (U/l)	5697±3697	5083±1344	-0.77 (0.134)	6403.9±2179	5298±1535	-2.30* (0.028)	6009±1749	5138±1242	-2.10* (0.033	6256±1805	4822±1671	-1.50 (0.137)	6073±2688	5155±1372	-3.04** (0.003)

n= number of persons; ALT =Serum Alanine transferase; AST= Aspartate Amino transferase; ALP= Alkaline phosphatase; *The mean difference was significant at the P<0.05 level; ** The mean difference was highly significant at the P<0.01 (t. test); AChES= Acetylcholinestrase activity in serum and Acetylcholinestrase activity in erythrocytes (AChER).

Table 4: Effect of chronic exposure to a mixture of pesticides on liver functions and acetylcholinestrase according to the number of pesticide applications

Parameters	Control	Exposed					
	Number of pesticide applications						
	0 (n=100)	10-50 (n=81)	*t (P)*	50-100 (n=13)	*t (P)*	Over 100 (n=6)	*t (P)*
ALT (U/l)	1.98±3.45	5.08±11.62	2.53* (0.012)	4.86±4.71	2.7** (0.007)	3.81±4.44	1.3 (0.219)
AST (U/l)	7.08±8.29	9.39±9.35	1.75* (0.030)	7.8±7.1	0.333 (0.366)	11.43±12.28	1.2 (0.228)
ALP (IU/l)	11.95±6.71	18.55±13.05	4.3* (0.047)	17.97±14.03	2.6** (0.011)	19.08±2.86	2.57** (0.011)
T. bilirubin (mg/dl)	0.52±.0.37	0.55±0.24	0.567 (0.571)	0.53±0.23	0.079 (0.112)	0.60±0.30	0.493 (0.623)
D. bilirubin (mg/dl)	0.24±0.20	0.04±0.01	-8.6** (0.001)	0.045±.018	-3.4**(0.001)	0.04±.02	-2.29* (0.024)
T. protein (g/dl)	8.52±1.44	7.38±1.57	-5.06** (0.002)	9.35±7.21	1.02 (0.307)	6.90±0.48	-2.73** (0.007)
AChES (IU/l)	5601±7522	4610±3973	-1.07 (0.286)	5055±2821	-0.25 (0.798)	2530±1221	-0.99 (0.322)
AChEE (IU/l)	6073±2688	5110±1345	-2.9** (0.007)	5774±1411	-0.39 (0.694)	4431±1369	-1.48 (0.142)

*The mean difference was significant at the P< 0.05 level (t. test); ** The mean difference was highly significant at the P<0.01 (t. test)

Table 5: Correlation between liver functions, total protein and AChE and Age in exposed and control groups

Parameters	Age (20 - 60 year)			
	Control	Sig	Exposed	Sig
AST (U/l)	-0.103	0.30	0.133	0.188
ALT (U/l)	-0.101	0.318	0.175	0.081
T. bili (mg/dl)	0.112	0.266	0.216*	0.031
D. bili (mg/dl)	-0.036	0.723	-0.036	0.042
ALP (IU/L)	0.307	0.002	-0.289**	0.004
T. protein (g/dl)	0.070	0.489	-0.046	0.648
AChES (IU/l)	-0.021	0.833	-0.220*	0.028
AChER (IU/l)	0.053	0.600	-0.033	0.747

*The mean difference was significant at the P< 0.05 level (t. test); ** The mean difference was highly significant at the P<0.01 (t. test)

Table 6: Correlation between liver functions, total protein and AChE and number of pesticides application

Parameters	Number of applications (10 <100 times)	
	Exposed	Sig
AST (U/l)	0.037	0.717
ALT (U/l)	-0.091	0.370
T. Bili (mg/dl)	0.035	0.730
D. Bili (mg/dl)	0.06	0.495
ALP (IU/L)	0.018	0.863
T. Protein (g/dl)	-0.020	0.842
AChES (IU/l)	-0.046	0.652
AChER (IU/l)	-0.034	0.739

4. Conclusions

The present study revealed that certain enzymes (AST, ALT, and ALP), as well as total protein, bilirubin and cholinesterase activity in serum and red blood cells, are to some extent influenced by pesticide exposure. Most farmers in our study were not aware of the health hazards caused by the inappropriate handling of pesticides. Awareness needs to be created on use of personal protective measures among farmers, while handling pesticides. Farmers needs to be encouraged to reduce, if not eliminate the use of pesticides, with the introduction of incentives to the farmers to help them shift from synthetic pesticides to bio-pesticides and organic farming.

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