Original Article

Evaluation of cholinesterase and lipid profile levels in chronic pesticide exposed persons

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Abstract

Background: Agriculture being the predominant occupation in India with rampant usage of pesticides to meet its enormous population needs. This pesticide abuse is taking a toll on the health of the persons involved in this work. **Objectives:** To evaluate pesticide exposure by assessing serum cholinesterase levels, and comparing them with the serum lipid profile levels which assumes the cardiovascular risk status. **Study Design:** It is a cross-sectional comparative study involving around 283 agricultural farm workers in Rajamahendravaram, Andhra Pradesh. The study period was for about 3 months during pesticide spraying season. **Materials and Methods:** All the blood samples were collected and analysed for biochemical parameters like plasma glucose, blood urea, serum creatinine, and lipid profile and serum cholinesterase levels using XL 640 fully automated random access analyser. **Statistical Analysis Used:** Results were analysed using SPSS software version 20. **Results:** The study group was classified into two groups based on serum cholinesterase levels. It was observed that there were significant alterations in lipid profile levels in the study group with decreased cholinesterase levels when compared to those of normal cholinesterase levels. There was significant negative correlation between cholinesterase levels and non-HDL cholesterol and total cholesterol/HDL ratio. **Conclusion:** This study implicates that the pesticides have an adverse health effect with regard to cardiovascular risk status.

Keywords: Cardiovascular risk, cholinesterase levels, lipid profile, pesticide exposure

Introduction

Pesticides are extensively used in the agriculture which is the predominant occupation in developing countries.[1] Farmers often use pesticides usually at higher concentrations than recommended.[2-4]

Despite the usage of such a large amount of pesticides, there is an estimated 10-30% loss due to pests alone. Organophosphates are irreversible inhibitors of cholinesterase resulting in impairment of metabolism of carbohydrates, fats and protein.[5-12]

The elevated levels of pesticide pollutants are associated with raised serum lipids which are a major risk factor for cardiovascular disease. If this association appears to be causal, it may have significant effects on human health.

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Materials and Methods

The study was conducted in the Department of Biochemistry, Andhra Pradesh, India, from the period April 2018 to July 2018. The study protocol was approved by the Institutional Research and Ethics Committee.

The study population comprised of farm workers using pesticides, and non-exposed controls from villages in Rajamahendravaram. The Sample size included 283 farm workers aged between 20-60 years. They were divided into two groups based on cholinesterase levels. Venous blood samples (about 5 ml) were taken by veni-puncture and the samples were left without anticoagulant to allow blood to clot. The serum samples were obtained by centrifugation at room temperature by Remi centrifuge at 4000 rpm/10 minutes. Serum samples were then used for bio-chemical analysis.

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Biochemical analysis

All the parameters were analysed using ERBA system pack reagents on Erba XL-640 fully automated analyser. Glucose was determined by using GOD-POD method, Urea by Urease-GLDH method, Creatinine by Enzymatic method, Cholinesterase by Spectrophotometric method, Cholesterol by CHOD-POD method, Triglycerides by GPO method and HDL by PVS/PEGME precipitation method.

Statistical analysis

Data were computer analysed using SPSS software version 20.0 and MS Excel-2007. The independent sample *t*-test procedure was used to compare different continuous variables between the two groups separated based on serum cholinesterase levels. Descriptive data was presented as mean ± standard deviation. The correlation was used to assess the relation between continuous variables. For all statistical analysis, $P < 0.05$ was considered as statistically significant.

Results

The mean values of the various biochemical parameters are shown in Table 1. Correlation between cholinesterase and other biochemical parameters are shown in Table 2. From Table1 it is seen that all lipid profile parameters showed significant increase in cases when compared to controls including the calculated parameters like non-HDL Cholesterol and cholesterol/HDL ratio except HDL cholesterol which showed significant decrease. Table 2 shows that there is a significant correlation between cholinesterase and other biochemical parameters like fasting plasma glucose, total cholesterol, non-HDL cholesterol and total cholesterol/HDL ratio.

Discussion

The role of pesticides in the development of diseases in humans is still controversial, despite their widespread use. Organophosphate (OP) and Carbamate insecticides form are the groups of chemicals that are mainly used in agriculture[13] which are replaced by organic pesticides like pyrethroids which can be exposed to in several ways such as breath, oral cavity, and skin.[14] Occupational exposure to pesticides is a possible risk factor for the development of chronic diseases in humans including diabetes and obesity.[15,16] The early life exposure to endocrine disrupting chemicals (EDC) such as insecticides has been associated with later life adversities such as obesity, diabetes and cancer. The mechanisms underlying such associations are unknown but are likely to be mediated by epigenetic changes such as DNA methylation and histone modifications.[17]

Table 1: Mean values of biochemical parameters in pesticide exposed worker as compared to control

Parameter	Cases		Controls		*P*
	Mean	Standard deviation	Mean	Standard deviation	
Fasting plasma glucose	101.68	43.22	96.58	40.60	0.545
Postprandial plasma glucose	150.60	70.77	147.52	66.98	0.820
Blood urea	23.50	7.346	21.20	5.81	0.086
Serum creatinine	0.864	0.153	0.802	0.149	0.043*
Total cholesterol	180.64	39.54	156.60	34.99	0.002*
Triglycerides	160.00	76.63	128.66	63.88	0.029*
HDL Cholesterol	39.12	7.98	42.74	9.60	0.043*
LDL Cholesterol	102.60	33.58	89.88	30.84	0.051*
Cholinesterase levels	3714.64	901.08	8086.36	1745.33	0.000*
Non-HDL Cholesterol	140.08	42.63	114.34	35.01	0.001*
Total cholesterol/HDL Cholesterol	4.7782	1.4753	3.8208	1.0922	0.000*
Age	43.14	7.94	42.32	11.23	0.820

*Significant

Table 2: Correlation of serum cholinesterase levels with other parameters in cases

Parameter	*r*	*P*
FBS	-0.367	0.009*
PPBS	-0.151	0.294
Urea	-0.077	0.594
Creatinine	0.136	0.346
Cholesterol	-0.367	0.009*
Triacylglycerol	0.006	0.966
HDL cholesterol	0.028	0.848
LDL Cholesterol	-0.232	0.105
Non-HDL Cholesterol	-0.520	0.000*
Total Cholesterol/HDL ratio	-0.301	0.034*

*Significant

In our study we divided the total study group into two based on cholinesterase levels. The group with decreased cholinesterase levels was considered as cases and normal cholinesterase levels as controls. The determination of cholinesterase activity has been used as a measure of exposure to organophosphates and carbamates and in the diagnosis of poisoning with anticholinesterase compounds. The strong lipophilic insecticides get incorporated in to bio membrane. Due to the crucial functional role of membranes in health, the insecticide effects are certainly membrane related. There are many studies in this aspect indicating the role of insecticide compounds in inducing perturbations of membrane permeability and enzyme dynamics (Antunes-Madeira and Madeira, 1979 and 1982; Antunes-Madeira *et al.*, 1981). Mainly membrane mechanisms are based on the membrane physical state and organization (Sikkema *et al.*, 1995). Hence the effects of insecticides are due to physical changes at the level of lipid-lipid and lipid-protein interaction. Partition studies by Antunes-Madeira and Madeira, 1989 state that membrane undergoes modulation for the incorporation of insecticide. Lipids undergo rapid breakdown, re-synthesis and inter conversion. It is essential to study various lipid fractions in different tissues to provide a clear picture of lipid metabolism in response to pesticides which we have not done in our study (Srinivasulu Reddy and Ramana Rao, 1989). In a study done by Ghosh and Chatterjee, 1989, where B.dissimilis was exposed to pesticides, they found that there is decrease in tissue lipid and proteins under pesticide stress. It could be due to several mechanisms viz., formation of lipoproteins which are utilized for repair of damaged cell and tissue organelles, direct utilization by cells for energy requirements, increased lipolysis, and damage to cellular organization.

Blood is an easily available fluid. So, we used it as an important diagnostic tool to assess toxicity of pesticides instead of tissue studies. Almost every living tissue is exposed to this fluid for exchange of material. Therefore, alteration of any component of blood can be assessed by evaluating serum biochemistry (Stonard, Evans 1999; Fetoui *et al.* 2008).

Regarding the blood glucose levels in our study, we measured only fasting and post prandial blood glucose levels which showed nothing significant change as shown in Table 1. But fasting blood glucose levels are more than normal reference level in cases when compared to controls which is not significant. An investigation of glucometabolic state in larger number of human subjects is necessary. There is growing evidence that prevalence of diabetes in newly diagnosed may be underestimated if FPG tests only had been performed.[18] In the Austrian study, 59% of patients with newly diagnosed AGR had fasting blood glucose levels below 5.5 mmol/L and so would have remained undiagnosed without the performance of OGTTs.[19] Even we did not perform OGTT in our study. So prevalence of diabetes could not be traced.

For the biochemical analysis of the sera, the BUN and creatinine which are primarily indicators of kidney function did not differ between the two groups though creatinine is shown as significantly elevated in cases the rise is within normal reference limit.

Fasting blood glucose levels showed significant negative correlation with cholinesterase levels which indicates some alteration in glucose metabolism. A study by Daniels SI *et al.* showed that South Asian immigrants have a higher body burden of organo chlorine pesticides than European whites. Diabetes mellitus is associated with higher pesticide concentrations in this population.[20] The toxicity of pesticides possibly is due to its stress-causing effect (Singh *et al.* 2009; Sadeghi-Hashjin *et al.* 2011). Stress conditions cause release of adrenocorticotropic hormone, triggering consequent secretion of cortisol by the adrenal cortex (Hayes, Laws 1991). The altered carbohydrate levels might be due to conversion to fats including triglycerides via intermediatory metabolism (Bhushan 2011; Guyton, Hall 2001). Phospholipids have both metabolic and structural function in mammals and are the main precursors of lipoproteins, the carriers for triglyceride transport (Zubay *et al.* 1995). Decreased serum cholinesterase activity (Kale *et al.* 1999), may also be responsible for enhanced serum phospholipid concentration. Increased serum cholesterol levels observed in the present study might be an outcome of cholestasis, along with endogenous synthesis of cholesterol (Saxena, Sharma 1999). Lack of study on parameters indicative of cholestasis might be a drawback for this study.

Xenobiotic substances activate the sympathetic nervous system, resulting in release of epinephrine and norepinephrine by adrenal medulla (Harrison 1994; Sadeghi-Hashjin *et al.* 2011). They activate hormone-sensitive triglyceride lipase in tissue, resulting in hydrolysis of stored triglycerides from fat stores and mobilization of free fatty acids in the blood stream causing raised serum total lipid concentration (Rani, Dua 1995; Guyton, Hall 2001). Free fatty acid estimation which is indicative of lipolysis was not done in this study.

From Table1, it is seen that in our study serum cholesterol, triglycerides and LDL levels had increased significantly in patients with decreased cholinesterase levels compared to the ones with normal cholinesterase levels. This indicates pesticides have effect on serum lipids. One of the causes of increased total lipid concentration appears to be disturbance of carbohydrate metabolism, due to probable cytotoxic effect on cells of the pancreas leading to relative deficiency of insulin (Kalender *et al.* 2005). In insulin deficiency, carbohydrates are not used as energy source and most of the energy is derived from fats. To meet the energy demands lipolysis occurs and the amount of free fatty acids in blood is increased, which we have not estimated in our study, resulting in increased serum total lipid concentration (Guyton, Hall 2001; Rezg *et al.* 2004).

For other markers of lipid metabolism, there were no effects of pesticides on high-density lipoprotein cholesterol (HDL-cholesterol), except one study reported negative correlation between organo chlorines and HDL[21] which corresponds to our study as HDL cholesterol levels were

decreased in cases when compared to controls. Others reported that pyrethroid were linked with disturbed lipid metabolism by increasing triglycerides, phospholipids, very low-density lipoprotein cholesterol (VLDLC), but no effects on HDL.[22] Hyperstimulation of the nervous system triggers energy demands resulting in the disorder of energy homeostasis that can lead to altered glucose and lipid metabolisms.[23-26]

There are many studies which state that elevated levels of persistent organic pollutants such as pesticides are associated with increased levels of serum lipids which are a major risk factor for cardiovascular disease.[1] If this association appears to be causal, it may have significant effects on human health. In our study, we have used non-HDL cholesterol and HDL/Total cholesterol ratio as the measurements tools for risk factor assessment for cardiovascular status. There are studies which show that chronic pesticide exposure leads to metabolic syndrome as shown by the one done by Mustieles V *et al.* This study shows that human adipose tissue levels of persistent organic pollutants results in metabolic syndrome as evidenced by having prevalence of more than one diagnosis of type 2 diabetes, hypertension, hypertriglyceridemia, and/or low HDL cholesterol in their study group.[27]

There are certain clinical trials on animal and humans in which they exposed the subjects to pesticides. In 1978 two laboratories reported that feeding rats with organo chlorines resulted in elevation of serum cholesterol.[2,3] Oda *et al.* reported that feeding rats with organic pollutants resulted in hypercholesterolemia which might be due to stimulation of de novo synthesis of liver lipids.[4] Azaïs-Braesco *et al.* studied the effects of two different PCB congeners and found that the changes in lipid profiles varied with congener structure.[10] Monkeys fed Aroclor 1254 developed elevated triglycerides, but decreased total cholesterol, HDL and LDL cholesterol.[11] Sanyal *et al.* reported elevations in lipid synthesis by the liver in DDT-treated monkeys.[12]

Another animal study on mice by Sun Q *et al.*, indicated that imidacloprid, a neonicotinoid insecticide, may potentiate high fat diet-induced adiposity in female C57BL/6J mice and enhance adipogenesis in 3T3-L1 adipocytes via the AMPKα-mediated pathway. Imidacloprid might also influence glucose homeostasis partially by inducing cellular oxidative stress in C2C12 myotubes.[28]

In experimental studies on humans, several authors have reported elevations in triglycerides[29,30] and total cholesterol.[31-33] Studies on occupationally exposed persons noted elevations in rates of cardiovascular deaths.[34] In a study on Native American population, they have noted that higher pesticide levels were associated with elevations of both triglycerides and total cholesterol which resulted in increased self-reported cardiovascular disease.[35] But the study was limited by the self-reported nature of the diagnosis. However in a study by General Electric scientists, they have argued that the elevated level of organophosphates is a consequence, not a cause, of the hyperlipidemia.[36,37] This is in contrast with our study which shows that the risk assessment by using HDL/total cholesterol ratio or non-HDL cholesterol levels negatively correlated with cholinesterase levels which is shown in Table 2.

Exposure to pesticides influences the genetic patterns of lipid metabolism.[38,39] This conclusion was made by an experimental animal study where they fed either an olive oil or a corn-oil rich diet, and then exposed to pesticides. They found that the pesticide exposure caused induction of genes involved in fatty acid degradation. Petriello *et al.* also showed that diets high in omega-6 fatty acids can worsen organophosphate-induced vascular toxicity while diets enriched with bioactive food components such as omega-3 polyunsaturated fatty acids can improve the toxicant-induced inflammation.[40]

The precise mechanisms underlying the associations between serum persistent organic pollutants and the various serum lipid components are uncertain. Organo chlorines exposure induces various degradative enzymes such as cytochrome P450s.[41,42] In addition; several persistent organic pollutants like chlorinated pesticides can alter DNA methylation levels which induce epigenetic changes.[43] Enzyme induction in highly exposed conditions can be identified by doubling of liver size in animals.[44] Other studies have shown that pesticide exposure can cause specific alternations of enzymes involved in lipid synthesis.[45] Enzyme alterations are dependent on variety of genes that are either up-or down-regulated.[46-49] Thus, there are multiple possible pathways that might lead to selective alterations in metabolism of body fat store that can be reflected in change of serum lipids.

Our results are consistent with the hypothesis that higher levels of persistent organic pollutants, especially higher organo chlorines, organophosphates or pyrethroids, result in elevations of serum lipids. The risk of cardiovascular disease is, at least in part, as a consequence of the elevations in serum lipids that result from exposure. There might be a component of abnormal blood glucose regulation which is inconclusive from this study. The results from cross-sectional study in Spain showed that persistent levels of pesticides were significantly higher among subjects having diabetes. These findings should be considered by public health authorities to implement measures devoted to minimize human exposure to pollutants that could be harmful to the population.[50] This study will help us to create awareness and implementation of the concept of safe occupational practice in agriculture farm workers in our country. The estimation of blood glucose and lipid profile play important role in the diagnosis of risk of coronary heart diseases in organophosphorus poisoning. Regular monitoring of Acetylcholinesterase (AChE) and Pseudocholinesterase in addition to effective interventions in regards to reducing pesticide exposure to prevent health effects should be provided to farmers.[51] Routine monitoring of AChE may allow for early recognition of chronic low-level exposure to organophosphates when they are in use by farmers. It is suggested that non farmers may also be exposed but are

less likely to regularly monitor their chemical exposure and use personal protective equipment when using chemicals.[52]

Conclusion

Our results are consistent with the hypothesis that higher levels of pesticides exposure for chronic period result in elevations of serum lipids and that risk of cardiovascular disease. Unsafe practices among agriculture workers cause significant decrease in cholinesterase levels and altered glucose metabolism. Hence farm workers need to practice basic personal protective equipment and regular health checkups to minimize adverse health effects.

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Conflicts of interest

There are no conflicts of interest.

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