

True and Pseudo Cholinesterase levels in short and long-term of pesticides exposures

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ABSTRACT

Aim: The present study is aimed to study and analyze the true and pseudo Cholinesterase levels in the subjects exposed to pesticides during the short term by intentional or accidental intake and long-term exposure due to their occupation. **Method:** 75 people were taken as controls who had no medical illness. A total number of 300 cases of pesticide poisoning were selected, aged between 20 to 55 years, which consist of 150 acute poisoning and 150 chronic poisoning subjects were taken as a case study, the detailed case history, and the type of organophosphorus pesticide taken were recorded. Whole blood cholinesterase levels and Pseudo cholinesterase levels were estimated. Mean and standard deviation (SD) of all variables were calculated and compared with those of controls. **Results:** During acute poisoning, the mean value of Whole blood cholinesterase/True cholinesterase (U/L) in acute poisoning cases on the 1st, 3rd, 7th, and 6months day was 1.27 ± 0.61 , 1.65 ± 0.65 , 2.221 ± 0.68 and 3.97 ± 0.4 respectively. The mean value of serum cholinesterase/pseudocholinesterase (U/L) in acute poisoning cases on the 1st, 3rd, 7th, and 6months 2213.05 ± 1749.81 , 2862.3 ± 2025.6 , 4008.4 ± 2355.9 and 7708.34 ± 880.72 respectively. During chronic poisoning exposure, the mean value of Whole blood cholinesterase (U/L) in controls is 4.0 ± 0.39 as compared to 3.019 ± 0.848 in cases of chronic poisoning. The difference between the study group and the control group was found to be statistically significant. The mean value of serum cholinesterase/pseudocholinesterase (U/L) in controls was 7991.97 ± 1276.5 as compared to 6214 ± 1189 in cases of chronic poisoning. The difference between the study group and the control group was found to be statistically significant. **Conclusion:** Thus, from this study, it can be concluded that the exposure to pesticides had detrimental effects on the health of the susceptible population, and choline Esterase enzyme is the Best marker to study and assess the pesticide exposure in the subjects exposed to pesticides.

KEYWORDS: True cholinesterase; Pseudo cholinesterase; Pesticide poisoning.

INTRODUCTION

Pesticides are widely used throughout the world in agriculture to protect crops and to safeguard the crop from damage. Tremendous amounts of pesticides are being used more particularly for growing paddy in south Indian states. Although it has beneficial effects on yield, lack of observance and unavailability of protective measures to counter the harmful effects of pesticides have posed a real threat to the health of farmers [1].

After India's Green Revolution started, the

consumption of pesticides in India has increased several hundred folds, from 154 MT in 1954 to 88,000 MT in 2000-2001 [2]. Pesticide poisoning among farmers in developing countries is alarming [3]. WHO had estimated approximately 20,000 workers die from exposure every year, and most of them are from developing countries? [4]. The number of intoxications with organophosphates is estimated at around 3000,000 per year [5].

Pesticide exposure may produce biochemical changes even before adverse clinical health effects are manifested in the farmers. Prolonged exposures to pesticides among farm workers

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affect multiple organs, including liver and kidney, which can be detected by serum enzymes and other biochemical parameters [6]. It is essential to identify the susceptible individuals among the farmers who are at risk of exposure to certain types of environmental and occupational agents [7]. Most toxic substances exert their effects on a fundamental level in the organism by reacting with enzymes or by affecting membranes and other functional components of the cells. Biochemical and physiological techniques are commonly used in the laboratories to measure them. Such effects of pesticide poisoning along with histological, histochemical, and hematological studies can contribute more fruitfully to reveal the toxic mechanism of a single or a group of substances [8].

AIM: To know the True and Pseudo Cholinesterase levels for analyzing the short and long-term toxic effects of pesticides.

MATERIAL AND METHODOLOGY

Study design: This is an observational, analytical study

Study period: 5 Years of a prospective study

Study location: Department of Biochemistry in collaboration with Medicine department, SVS Medical College, Yenugonda, Mahabubnagar district

Ethical approval: The study was approved by the Institutional ethics committee and carried out as a part of a Ph.D. study under Dr. NTR University of health sciences. Informed consent was obtained from participants or family members.

Sample size: 300 cases of pesticide poisoning {150 acute and 150 chronic poisonings} subjects and 75 controls (n=375)

Inclusion criteria: Age group between 20-55 years, and both sexes were included.

Control: Normal healthy persons, i.e., no history of diabetes, hypertension, alcoholism, and smoking.

Test group: Farmers who are occupationally involved in pesticide handling/ spraying, for analyzing the long-term (more than five years) effects of pesticides. (Group 1: Chronic). Patients admitted to the hospital for acute poisoning with a definite history of intake of pesticides. (Intentional/accidental) or inhalation for analyzing the short-term effects (group 2: Acute).

Exclusion criteria: Patients having a history of diabetes and hypertension and other endocrine disorders.

Methodology:

A questionnaire was designed on this subject to gather the necessary information related to their health, which may be useful in evaluating their health status. Data was collected through a survey in the field by face-to-face interviews with farmers/farm workers and field observations during farming activities. The questionnaire was the regional language (Telugu/Urdu). The questionnaire consisted of data as age, marital status, educational status, family data, and history of illness suffered and history of acute pesticide poisoning e.t.c.

Collection of blood: A venous blood sample was drawn from the participants into a sterile disposable syringe, which was transferred into both heparinized and nonheparinized tubes and was centrifuged at 3000rpm for 10 minutes. The Serum/Plasma/R.B.C/W.B.C was separated and collected and analyzed as follows. The samples were refrigerated at 0-40 C for any future studies. In acute cases, the estimations are repeated at 1st, 3rd, 7th day, and the end of the 6months. All acute poisoning cases are treated by standard procedures.

Biochemical analysis: Whole blood cholinesterase levels: It was estimated by the kinetic method using Systronics UV-V7S-Spectrophotometer 117 [9-10]. The rate of hydrolysis of acetylcholine by a red cell suspension at pH 7.2 is measured at 412 nm by the reaction of thiocholine with DTNB to give the yellow 5-thio-2-nitrobenzoate anion (molar absorptivity, 13.6×10^6 L/mol/cm).

Pseudo cholinesterase levels: Kinetic method using evolution 3000-Tulip Semi auto analyzer. [11]. Butyrylthiocholine is hydrolyzed by cholinesterase to produce thiocholine in the presence of potassium hexacyanoferrate (III); the absorbance decrease is proportional to the cholinesterase activity of the sample.

Statistical Analysis: Data was analyzed by using statistical package for social sciences(SPSS) version 16.0. The comparisons between groups are done by using ANOVA(Analysis of variance test) for continuous normal data, and continuous non-normal data was done by Kruskal's walli's test followed by multiple comparisons was analyzed by Dunnett's/Dunn's test. The comparison for two groups was made by unpaired t-test for continuous normal data, and Mann Whitney U-test was applied for continuous non-normal data. All P-values less than 0.05 were considered statistically significant.

RESULTS

Table 1. The comparison between controls and farmers

Parameters	Groups	N	Range	Median (IQR)	p-value
RBC cholinesterase (3-8 U/ml)	Controls	75	3.4 -4.7	4.06 (3.9-4.2)	0.001
	Farmers	150	1.7-4.6	2.9 (2.3-3.9)	
Serum Cholinesterase (4850 – 9000 U/L)	Controls	75	5496-9894	8260 (6956-9894)	0.001
	Farmers	150	3870-8840	6230 (5420-6948)	

Table2. The comparison between controls and hospital cases (Group 2: Acute)

Parameter		Control	Cases (n=150)			
			Day 1	Days 3	Day 7	6 months
R B C cholinesterase (3-8 U/ml)	Range	3.4-4.7	0.38-2.9	0.6-3.1	0.9-3.6	3.2-4.6
	Median	4.0 (3.9-4.2)	1.26 (0.4-2.9)	1.65 (1.2-2.2)	2.22 (1.6-2.8)	4.1 (3.6-4.2)
	P-value		0.0001	0.0001	0.0001	N.S
Serum Cholinesterase (4850 – 9000 U/L)	Range	5496-9894	436-4456	683-9160	1100-9990	5675-9214
	Median	8260 (6956-9894)	1709 (793.8-3083)	2061 (1400-3869)	3132 (2095-5658)	7739 (7047-8320)
	P-value	-	0.0001	0.0001	0.0001	N.S

*P-value: Comparison with the control group, NS: Non-Significant

Table 3. The comparison between 6 months of acute exposure subjects and farmers

Parameters	Groups	N	Range	Median (IQR)	p-value
RBC cholinesterase (3-8 U/ml)	Acute	50 ^s	3.2-4.6	4.1 (3.6-4.2)	0.001
	Chronic	150	1.7-4.6	2.900 (2.3-3.9)	
Serum Cholinesterase (4850 – 9000 U/L)	Acute	50 ^s	5675-9214	7739 (7047-8320)	0.001
	Chronic	150	3870-8840	6230 (5420-6948)	

^sThe acute poisoning patients who were discharged from the hospital when contacted for screening after six months, only 50 accepted to get tested out of 150 who were discharged from the hospital.

DISCUSSION

The primary mechanism of action of organophosphorus pesticides is inhibition of acetylcholinesterase. Acetylcholinesterase

is an enzyme found on red blood cell (RBC) membranes that degrades the neurotransmitter acetylcholine (ACh) into choline and acetic acid. Acetylcholine is in the central and peripheral nervous system, neuromuscular junctions, and red blood cells. Pseudo cholinesterase, which is a liver acute phase protein present in plasma and nervous tissue, is inhibited by organophosphorus compounds in a similar way to acetylcholinesterase. Still, the specificity of the two enzymes is different. Organophosphates inactivate acetylcholinesterase by phosphorylating the serine hydroxyl group located at the active site of acetylcholinesterase. The phosphorylation occurs by the loss of an organophosphate group and the establishment of a covalent bond with acetylcholinesterase. Once acetylcholinesterase has been inactivated, acetylcholine accumulates throughout the nervous system, resulting in overstimulation of muscarinic and nicotinic receptors. Clinical effects are manifested via activation of the autonomic and central nervous systems and at nicotinic receptors on skeletal muscle. Butryl cholinesterase, like other serine esterases, reacts with organophosphorus compounds forming phosphorylated esterase's [12]. Depending upon the toxicity of the compound and exposure time, the symptoms of pesticide exposure vary from headache, vomiting, skin rash, respiratory problems, convulsions, and sometimes death may occur if untreated [13]. Many people are continually exposed to low OP concentrations, and long-term epidemiologic studies reveal linkage to a higher risk of cancer development [14-15]. In the present study, we found that the incidence of poisoning was more common among the age group between 15 to 40 years, and the organophosphorus compounds the most commonly used spraying and suicidal agents were Monocrotophos, Chlorpyrifos, Dimethoate, prophenophos, Profenofos, and Endosulfan. The present study supports and correlates with the above study where RBC cholinesterase and serum cholinesterase activity were decreased significantly both in group 1 and as well as in group 2 when compared with controls.

CONCLUSION

From the present study, it can be concluded that RBC cholinesterase and serum cholinesterase activity in all the cases was decreased significantly both in acute and as well as in chronic poisoning. But it is interesting to observe that the acute poisoning patients who were discharged from the hospital showed recovery in both the cholinesterase levels after six months which may be attributed to the effective treatment undergone by this cases wile admitted in the hospital, but the cholinesterase

levels were found to be significantly decreased in chronically exposed farmers when compared with controls and the six months values of acute poisoning cases, which is a matter of concern showing there is a persistent effect of organophosphorus compounds present in the farmers. It can be further concluded that the blood cholinesterase levels are the hallmark of pesticide poisoning even in chronically exposed subjects.

Conflict of interest : Nil

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