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ORIGINAL ARTICLE

Acute Poisoning with Organophosphorus Pesticide: Patients Admitted to A Hospital in Bijapur, Karnataka.

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Abstract:

Background: Organophosphorus compounds are the pesticides most often involved in human poisoning. Toxicity of these compounds is due to the inhibition of acetyl cholinesterase at cholinergic junctions of the nervous system. *Aims & Objectives:* Toxicities of OP pesticides cause adverse effects on many organs and systems hence the present study was planned to study the plasma Cholinesterase, serum cholesterol and thyroid function tests in acute organophosphorus pesticide poisoning. *Materials and Methods:* Plasma ChE, serum cholesterol and serum triiodothyronine, thyroxine, and thyroid stimulating hormone levels were estimated using standard methods. *Result:* In our study we found the maximum (95%) cases were suicidal poisoning. We found that the incidence of poisoning was more common among age group between 15-35 years and males (57%) were more likely to attempt suicide as compared to females (38%). Among the organophosphorus compounds the most commonly used were dimethoate, monocrotophos, chlorpyriphos. 79% organophosphorus poisoned patients recovered, while 21% died. Plasma Cholinesterase levels were significantly ($p<0.001$) decreased in all grades of organophosphate poisoning as compared to controls. Inhibition of Plasma Cholinesterase occurs at the time of admission due to toxic effect of organophosphorus compounds; but the levels significantly ($p<0.001$) normalized after treatment i.e. on the last day of hospitalization. Serum total cholesterol levels significantly decreased ($p<0.001$) in all grades of organophosphate poisoning cases as compared to controls without any change after treatment as compared with the patients before treatment. There was a slight and nonsignificant decrease in serum triiodothyronine and serum thyroxine levels in organophosphorus poisoning cases without any significant change in serum thyroid stimulating hormone levels as compared to control. The organophosphorus poisoned patients after treatment do not show more changes in their values. *Conclusions:* The present finding indicate that plasma ChE can be used as a parameter to assess the severity of poisoning and also to monitor the prognosis of OP poisoning. We also support that serum cholesterol estimation can be used as a biological marker in intentional OP poisoning. Acute OP poisoning may not disrupt thyroid hormone metabolism.

Key Words: Cholinesterase (ChE), Organophosphorus (OP) poisoning,

Triiodothyronine (T3), Thyroxine (T4), and Thyroid Stimulating Hormone (TSH).

Introduction:

Organophosphates are used extensively in India to control malaria and to increase production of agricultural commodities. These chemicals are beneficial to mankind due to their toxic properties but they also pose a risk to humans [1]. Hundreds of organophosphates are currently available for use as pesticides. It is estimated that 90% of fatal pesticide poisoning occurs in developing countries [2]. Use of pesticide as a mean to end the life is grouped under nonviolent methods for suicide. As agriculture is the main occupation in developing countries; pesticides are easily available in lethal and concentrated forms.

Self poisoning with organophosphorus (OP) compounds is a major global problem associated with thousands of deaths every year. Unemployment, failure in examination, social and domestic as well as economic problems are increasing; ultimately creating psychological disturbances. Such stress may force a person to consume poisons such as OP compounds due to its low price, high toxicity and easy availability. Hence these compounds are most often misused for suicidal purposes in our country. Since 1963 there has been a steady increase in the incidence of organophosphorus poisoning cases in India [1]. Accidental OP poisoning may occur due to inhalation while spraying the crops. Self poisoning is always by ingestion for committing suicide.

Pesticides are neurotoxins that can cause acute symptoms as well as chronic effects from repeated low-dose exposure. These compounds can adversely affect the immune system; causing cell mediated immune deficiency, allergy and auto immunity [3].

Clinical manifestations of OP poisoning are caused by excessive synaptic accumulation of Acetylcholine (ACh). Organophosphorus compounds irreversibly inhibit the enzyme Acetylcholine estrase (AChE) resulting in excessive accumulation of Acetylcholine, leading to the paralysis of cholinergic transmission in the central nervous system, autonomic ganglia, parasympathetic nerve endings, some sympathetic nerve endings and neuromuscular junctions [4]. The leading cause of death in OP poisoning is respiratory failure which results from a combination of respiratory muscle weakness; central respiratory depression, increased bronchial secretions, bronchospasm and pulmonary edema [5]. The mortality rate of acute OP poisoning depends on the type of compound used, amount ingested, general health of the patient and delayed diagnosis and treatment [6]. Toxicity of OP compounds causes oxidative damage of cell membranes and also results in disturbed biochemical and physiological functions. Hence the present study was planned to study the plasma ChE, serum cholesterol and thyroid function tests in acute organophosphorus pesticide poisoning.

Materials and Methods:

The present study was carried out in the Department of Biochemistry; Bijapur Liberal District Educations University, Shri. B. M. Patil Medical College, Hospital and Research

Centre. The study was approved by the ethical committee. It included 150 organophosphate poisoning patients and 30 age and sex matched healthy controls. We also studied 40 OP poisoning cases at the time of admission to the hospital / before treatment and on the last day of hospitalization after discharge due to recovery. Diagnosis was based on information taken both from the patient or the patient's family and characteristic clinical signs and symptoms. Clinically diagnosed OP poisoning cases on the basis of definite history by patient or attendants and also confirmed by clinical features and characteristic odour of stomach wash or vomitus were included in the study. Patients with double poisoning, chronic lung disease, pulmonary tuberculosis, renal failure, known neuromuscular disease, smokers and alcoholics which may interfere in assessment of oxidative stress; were excluded from the study.

The grouping of the OP poisoning cases was done depending upon signs and symptoms.

Grade I - OP poisoned with no signs and symptoms (n = 26).

Grade II - Diarrhoea, vomiting, abdominal pain, giddiness (n = 27).

Grade III - Pupillary constriction with above symptoms (n = 31).

Grade IV - Pulmonary edema with above symptoms (n = 34).

Grade V - Unconsciousness with above symptoms (n = 32).

Immediately after admission to the hospital, before starting the appropriate treatment; 10 ml venous blood sample was collected from each of the subjects under aseptic conditions. Out of this 4 ml blood was transferred to heparinised bulb and 6 ml to plain bulb. Serum and plasma were separated by centrifugation at 3,000 rpm for 10 minutes, at room temperature. Then all samples were immediately placed at 4^0 C until they were processed to get accurate and reproducible results. After separation of the plasma, erythrocytes were washed three times with 0.9% saline solution. To one volume of washed erythrocytes nine volumes of cold redistilled water was mixed then centrifuged for 10 minutes to get the hemolysate which was used for estimation of erythrocyte Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx).

The plasma ChE was estimated by Butyrylthiocholine Kinetic Method, using standard kit of Agappe Diagnostics. Cholinesterase acts on butyrylthiocholine to form thiocholine which acts on dithio-bis-nitro benzoic acid giving pink colored 2-nitro, 5-mercaptobenzoate [7, 8]. Serum cholesterol was assessed by CHOD-PAP method, using standard kit of Agappe Diagnostics. Cholesterol esterase hydrolyses cholesterol esters into free cholesterol and fatty acids. Cholesterol oxidase converts cholesterol to cholest–4–en-3-one and H_2O_2. In presence of peroxidase, H_2O_2 oxidatively couples with 4–aminoantipyrine and phenol to produce red coloured quinoneimine dye which has absorbance maximum at 510 nm. The intensity of red colour is proportional to the amount of total cholesterol present in the specimen [9]. Estimation of Triiodothyronine (T3), Thyroxine (T4), and Thyroid Stimulating Hormone (TSH) levels were carried out by enzyme-linked immunosorbent assay which

were done at Thyrocare Laboratory, Mumbai. Statistical analysis of the data was performed using student "*t*" test. The values were expressed as mean ± SD. P value less than 0.05 ($p<0.05$) was considered as statistically significant.

Results and Discussion:

Organophosphorus poisoning is the most common cause of suicidal deaths in India. The incidents of poisoning are very common in individuals with low economic status. We found that the maximum cases (143/150 i.e. 95.33%) were suicidal poisoning as shown in

Table-1: Nature of poisoning

Nature of poisoning	Male No	Female No	Total No	Total %
Suicidal	86	57	143	95.33
Accidental	5	1	6	4.0
Occupational	1	-	1	0.67
Total	**92**	**58**	**150**	**100**

(Table-1).

It might be due to rapid urbanization, social and economic factors which mainly contribute to frustration and depression in the people. The persons who are not able to cope up with these stressful situations are the major victims of suicidal poisoning. Choice of OP compounds for suicide is mainly due to known toxicity of these agents and also cheap and easy availability of these compounds over the counter. In our study we found that males (57.33%) were more likely to attempt suicide than females (38%). The incidence of poisoning was more common among the age groups between 15 to 35 years. (Table-2). This is the most critical period, when one is likely to face various problems that may lead to psychological stress so a person may take drastic steps to end his life, consuming available poisons.

Table-2: Age wise Distribution of total subjects in the study group

Age in years	Male		Female	
	No	Percentage	No	Percentage
15-25	33	22	28	18.67
25-35	39	26	19	12.67
35-45	20	13.33	11	7.33
Total	**92**	**61.33**	**58**	**38.67**

Among the OP compounds the most commonly used compounds for suicidal purpose or in accidental poisoing in our study were dimetoate, monocrotophos, chlorpyriphos, paraoxan,

Table-3: Types of OP Compounds consumed for suicidal purpose or accidentally

Name of OP compound	No. of cases	%
Chlorpyriphos	17	11.33
Monocrotophos	19	12.66
Dimethoate	48	32.0
Mevinphos	13	8.66
Paraoxan	15	10.0
Metacid	14	9.33
Dicrotophos	8	5.33
Cypermethrin	4	2.66
Dichlorvos	3	2.0
Quinolphos	3	2.0
Metacol	5	3.33
Triazophos	1	0.67
Total	**150**	**100**

mevinphos, triazophos as shown in (Table-3). We found that 79.33% recovered, while 20.67% died, mainly due to respiratory failure. Number of death were more in grade IV and V (Table-4).

Table-4: Clinical outcome in the study group

Nature of poisoning	Total no. of cases	Recovered		Recovered	
		No. of cases	%	No. of cases	%
Grade I	26	25	96.15	1	3.84
Grade II	27	24	88.88	3	11.11
Grade III	31	26	83.87	5	16.12
Grade IV	34	25	73.53	9	26.47
Grade V	32	19	59.37	13	40.62
Total	**150**	**119**	**79.33**	**31**	**20.67**

Most of the patients were agricultural workers (31.33%), labourer (20.67%) and housewives (19.33%). It might be due to easy availability and accessibility of the pesticides among them (Fig. 1). The plasma ChE level of normal healthy control group ranged from 4500-8000 U/L and in OP poisoning cases 400-4800 U/L. We found significant ($P < 0.001$) and progressive decline in plasma ChE levels in all grades of OP poisoning cases compared to controls. Further with an increase in the severity of poisoning there was corresponding decrease in plasma ChE activities as shown in (Table-5) and Fig. 2.

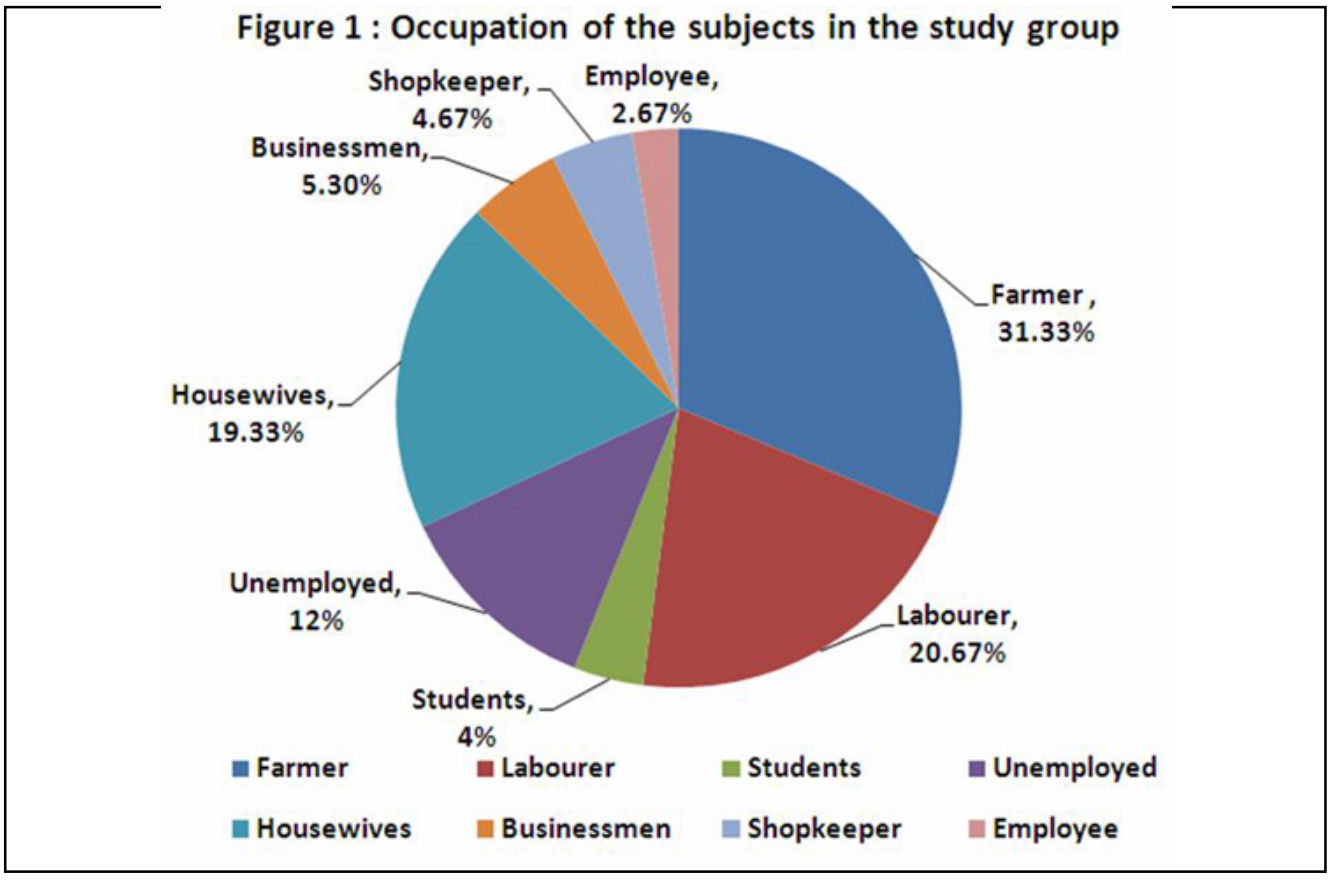


Table-5: Mean ± SD values of Plasma Cholinesterase (ChE) and Serum cholesterol levels in controls and different grades of OP poisoning cases

Study Groups	Plasma ChE (U/L)	Serum Cholesterol (mg/dl)
Controls (N=30)	6286 ± 912.53	210.13 ± 15.60
Grade I (N=26)	4586.4 ± 259.9**	154.75 ± 20.52**
Grade II (N=27)	3506 ± 234.7**	148.02 ± 19.2**
Grade III (N=31)	2462 ± 239.2**	142.12 ± 17.1**
Grade IV (N=34)	1390 ± 254**	132.42 ± 20**
Grade V (N=32)	614 ± 211**	136 ± 16.1**

** indicates P< 0.001 – highly significant

Significant depression in plasma ChE activity suggests that estimation of plasma ChE is useful in confirmation of OP poisoning. The results were consistent with earlier studies [10-12]. While no correlation between serum ChE level and severity of OP poisoning has also been reported [13].

Significant increase ($p<0.001$) in ChE levels after the treatment as compared with the OP poisoning patients before treatment is suggestive of clinical improvement (Table-6). It has been well established that the therapy with atropine and PAM causes restoration of ChE activity which occurs due to *de-novo* synthesis of fresh enzyme and also to some extent as a result of spontaneous dephosphorylation of the inhibited enzyme [14]. If the levels do not

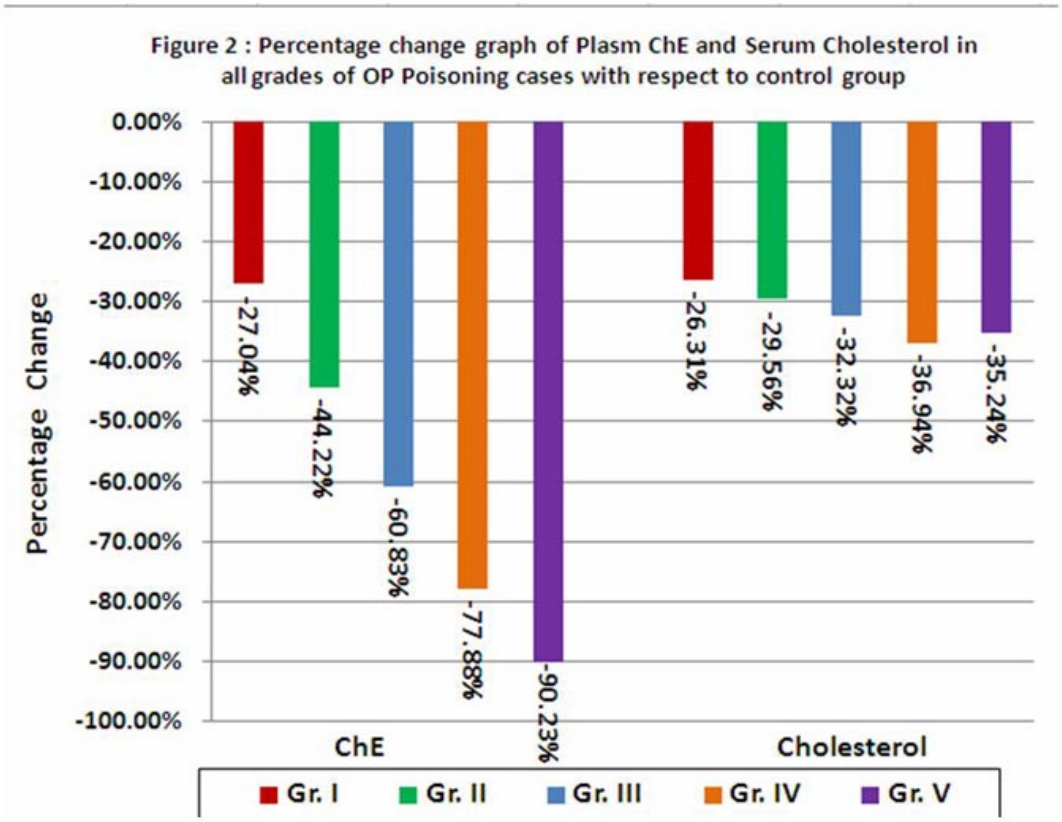


Table-6: Mean ± SD values of plasma ChE, serum cholesterol and serum T_3, T_4, TSH in controls and OP poisoning cases before and after treatment

Parameters	Controls (n=30)	OP poisoned cases	
		Before Treatment (n=30)	After Treatment (n=30)
ChE (U/L)	6286 ± 912.53	2015 ± 1040**	3880 ± 569**
Chol (mg/dl)	210.13 ± 15.60	136.44 ± 17.2**	136.88 ± 11.30(NS)
T3 (ng/dl)	108.1 ± 17.25	104.4 ± 16.7 (NS)	105.2 ± 19.5 (NS)
T4 (µg/dl)	10.25 ± 2.02	9.99 ± 1.09 (NS)	9.75 ± 1.54 (NS)
TSH (µIU/ml)	1.22 ± 0.78	1.46 ± 0.75 (NS)	1.42 ± 1.05 (NS)

** indicates P< 0.001 – highly significant, NS indicates nonsignificant

increase, excessive ACh accumulates at synapses within sympathetic ganglia and skeletal myoneural junctions. The increase in ChE enzyme parallels regeneration of true ChE and serves as an indicator of clinical improvement of the patient. Recovery after an acute episode of OP poisoning usually completes within 7 days unless anoxia has occurred during acute phase of the episode [15]. ChE returns to normal within few weeks because it is rapidly replaced by new enzyme synthesized in liver [16]. Hence plasma ChE could be used as a parameter to monitor the prognosis of OP poisoning. Chances of recovery are greater when the patient is hospitalized at the earliest indication of poisoning [17, 18].

Serum total cholesterol levels have significantly decreased (P< 0.001) in all grades of OP poisoning cases when compared to controls (Table-5 and Fig. 2) without any change after treatment as compared with the patients before treatment (Table-7).

Lowered serum cholesterol may decrease brain cell membrane cholesterol, lower the lipid microviscocity and decrease the exposure of protein serotonin receptors on the membrane surface, resulting in poorer uptake of serotonin from the blood and less serotonin entry into brain cell. The lowered cholesterol may affect brain serotonin enough to trigger violence or suicide in susceptible adults prone to such behaviour [19]. Direct relationship between low cholesterol level and suicidal behaviour in mentally ill patient was reported [20].

Another explanation is that most of the subjects in this study belong to lower socioeconomic background; hence the diet may be of low lipid content along with deficiency of high class protein. Tryptophan is an essential amino acid which is the precursor of serotonin; a neurotransmitter whose deficiency is known to induce depression, suicidal tendencies and aggressive behaviour [21].

Reduced levels of total cholesterol and phospholipids in humans in-vitro study due to

OP compound was also been reported [22]. The oxidative stress and modification of cellular membrane lipids may be involved in altering the function of membrane bound enzymes which may have serious consequences on neuronal functioning. Hence we support that it can be used as a biological marker in intentional OP poisoning.

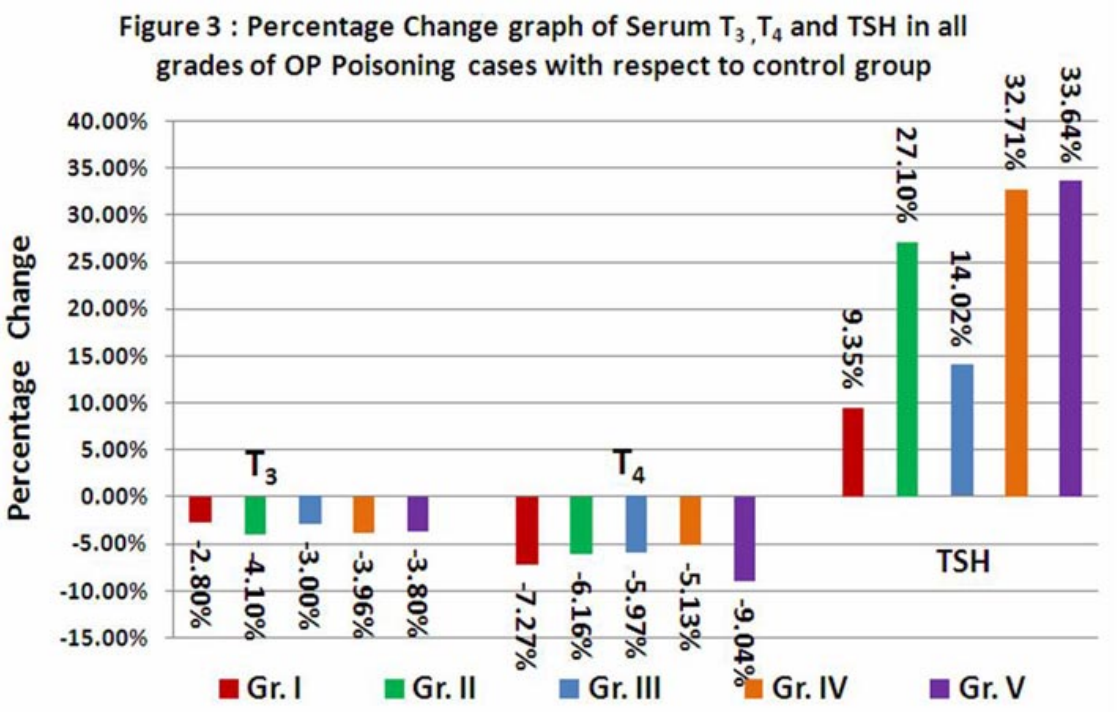
There was a slight and nonsignificant decrease in serum T3 and serum T4 levels in OP poisoning cases without any significant change in serum TSH levels as compared to control (Table-7 and Fig. 3).

The OP poisoned patients after treatment do not

Table-7: Mean ± SD values of serum T_3, T_4, TSH in controls and different grades of OP poisoning cases

Study Groups	T_3 (ng/dl)	T_4 (µg/dl)	TSH (µIU/ml)
Controls (N=30)	108.1 ± 17.25	10.25 ± 2.02	1.22 ± 0.78
Grade I (N=26)	105.02 ± 16.51•	9.94 ± 2.08•	1.17 ± 0.84•
Grade II (N=27)	103.65 ± 17.8•	10.06 ± 2.12•	1.36 ± 0.86•
Grade III (N=31)	104.82 ± 16.8•	10.08 ± 1.99•	1.22 ± 0.94•
Grade IV (N=34)	103.8 ± 15.5•	9.95 ± 1.91•	1.42 ± 0.96•
Grade V (N=32)	104 ± 19.81•	10.17 ± 1.65•	1.33 ± 1.08•

• Indicates P > 0.05 – non significant.



show more changes in their values (Table-6). Sarah Myhill suggested a suppression of the pituitary gland with borderline under activity of the thyroid (hypothyroidism) in pesticide poisoning (23). Our findings indicate that acute OP poisoning may not disrupt thyroid hormone metabolism.

Acute pesticide poisoning is now an important cause of morbidity and mortality worldwide. Due to lack of official guidelines about the usage of pesticides in agriculture & domestic life there is a surge in the misuse of these agents in developing countries like India. Mortality due to OP poisoning can be prevented with assessment of severity and timely institutional care. Plasma ChE estimation can be used as a parameter to assess the severity of poisoning & also to monitor the prognosis of OP poisoning. We also support that serum cholesterol estimation can be used as a biological marker in intentional OP poisoning. Acute OP poisoning may not disrupt thyroid hormone metabolism.

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