Original Article

Effects of acute organophosphate poisoning on pituitary target gland hormones at admission, discharge and three months after poisoning: A hospital based pilot study

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A B S T R A C T

Background: Organophosphate compound (OPC) poisoning is common in the developing countries such as India. The acute and later effects of OPC poisoning on pituitary and target gland hormones is largely unknown. **Materials and Methods:** This prospective study was conducted at Postgraduate Institute of Medical Education and Research between January 2012 and March 2013. Fourteen patients (8 males, age 18-50 years) with acute OPC poisoning were included in the study based on the history and clinical features, documented decreased in plasma cholinesterase activity or presence of the OPC in gastric lavage/blood samples. The hormonal parameters were done at baseline, at the time of discharge and at three months of follow-up. **Results:** A total of 14 patients out of 46 with the mean age of 30.1 ± 10.3 years were finally eligible for the study. Hormonal alterations at admission were similar to sick euhormonal syndrome. Overall 7 of them had nine hormonal deficits at three months of follow up, 4 having sub normal basal cortisol level and two each had low testosterone and growth hormone and only one had thyroxine deficiency. **Conclusion:** Acute organophosphate poisoning results in endocrine dysfunction akin to sick euhormonal syndrome. However, in a small subset of patients, varying level of hormonal insufficiency may occur either at admission or later. These observations need re-validation in a larger group of patients with specific OPC.

Key words: Endocrine dysfunction, organophosphate poisoning, pituitary

INTRODUCTION

Organophosphate compounds (OPCs) are widely used for pest control in agriculture worldwide which leads to increased risk of human exposure.[1-5] As a result of easy and widespread availability they are also often used as suicidal agents in developing countries killing an estimated 300,000 people per annum.[3,4]

The OPCs inhibit the acetyl cholinesterase (AChE) at nerve synapses leading to accumulation of acetylcholine (ACh) at neuromuscular junctions causing over-stimulation of ACh receptors with varied clinical manifestations.[4] Many afferent neural pathways converge from different parts of the brain onto the hypothalamus. Neurotransmitters secreted by these neurons modulate pituitary hormone secretion.[6-8] The cholinesterase inhibitors cross the blood brain barrier and affect the acetylcholine receptors in the brain. Critical illness and several drugs can also alter the functions of neurotransmitters and affect the release of pituitary hormones.[9-11] Several studies have used both cholinergic agonists and antagonists to demonstrate their effect on these receptors.[6-8,12]

Effects of OPCs on cardiovascular, nervous and respiratory systems are well known but their effect on endocrine system

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is not properly studied.[13-16] Hence, this study was planned to evaluate alterations in pituitary-target gland hormones at admission, at discharge following recovery and at three months of follow-up after acute OPC poisoning.

MATERIALS AND METHODS

Patient selection

This prospective study was conducted in patients with OPC poisoning admitted at Post graduate Institute of Medical Education and Research, Chandigarh, India between January 2012 and March 2013. Of the 46 patients admitted with suspected poisoning, 16 met the inclusion criteria (age range 18-50 years) with the history and clinical features consistent with OPC consumption, documented decrease in plasma cholinesterase activity or presence of the OPC in gastric lavage or blood [Figure 1]. Patients with the history of consumption of any compound other than OPCs, on antidote therapy before hospital admission, prior known endocrine disorder or on any hormone replacements, and pregnant/lactating females were excluded from the study. The study was approved by the Institute's Ethics Committee and Informed consent was taken from all the subjects.

Laboratory methods

Venous samples were collected in each patient at admission prior to initiation of treatment, at discharge and 3 months of follow up. Blood samples were analyzed for plasma cholinesterase and pre-treatment levels of serum Adenocorticotrphic hormone (ACTH), Cortisol, Dehydroepiandrosterone sulphate (DHEA-S), Prolactin (PRL), Leutinizing hormone (LH), Follicular stimulating hormone (FSH), Triidothyronine (T_3), Thyroxine (T_4), thyroid stimulating hormone (TSH), Insulin like growth factor-1 (IGF-1), Estradiol (E2, in females),

testosterone (T, in males). Free triidothyronine (FT3) and Free thyroxine (FT4) were estimated form the blood only if required. Serum ACTH, cortisol, DHEA-S, PRL, LH, FSH, T_3, T_4, TSH, E2 and T were estimated by electro chemiluminescence immune-assay (ECLIA) using the analyzer ELECSYS 2010 (Roche, Germany). IGF-1 (150-350 ng/ml) levels were assayed using the DRG IGF-1 600 ELISA kit, catalogue# EIA-4140. Patients in whom there was sub normal level of IGF-1/GH and cortisol at third month of follow up were subjected to insulin induced hypoglycaemia (IIH) to check the integrity of the hypothalamo-pituitary axis. IIH was performed by injecting 0.05 to 0.15 units of regular insulin intravenously. Venous blood samples were analysed for glucose, growth hormone and cortisol at 0, 30, 60, 90 and 120 min. Hypoglycaemia was considered as significant only if plasma glucose was <40 mg/dl along with signs and symptoms. The point of occurrence of documented hypoglycaemia was considered as 30 min sample A GH cut off of more than 5 ng/ml and cortisol cut off of 540 nmol/L were considered normal response to IIH. All study samples for specific hormones were processed in a single assay to avoid inter assay variability. The inter and intra assay coefficient of variation was <5%.

Gastric lavage analysis

Nasogastric tube was inserted in all the patients as soon as they were admitted in the hospital for the diagnostic and therapeutic purpose. The gastric lavage was analysed for the type of OPC. The serum cholinesterase was assayed by the method described as per Ellman *et al.*[17] Levels less than 1.2 U/L were considered diagnostic for OPC poisoning. Qualitative test for OPC in stomach contents was based on the Basic Analytical Toxicology by WHO under International Programme on Chemical Safety by silica gel thin layer chromatography plate with sensitivity to detect 5mg OPC per litre.[17,18]

Statistical analysis

The data was analyzed using Minitab 16. The normalcy of data was checked by Kolmogorov-Smirnov test. The mean and median of the parameters were obtained and they were compared for any significant difference in the following manner: Before treatment and at discharge; at discharge and at 3 months of OPC poisoning; and before treatment and at 3 months of follow up. Categorical variables were presented in percentages and tested for difference using Chi square test and Fisher's exact test. Continuous variables were analyzed depending on whether data was normal in distribution. Friedman's test with post-Hoc Bonferroni's correction was used for non-parametric variables whereas repeated measures ANOVA with post-Hoc Bonferroni's correction for parametric data. Significance was set at $P \leq 0.05$.
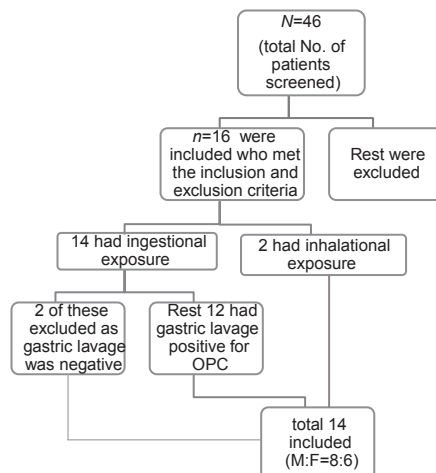

Figure 1: Concert of patient flow

RESULTS

The study group initially included 18 patients admitted to the hospital in the stipulated period. Sixteen patients had history of ingestion of OPC and two had inhalational exposure. Of 16 patients with OPC injestion, two were excluded as their gastric lavage did not show any evidence of OPC. Finally, eight males and six females were enrolled in the study [Figure 1].

The mean age, duration of hospital stay and Glasgow Coma Scale (GCS) of the subjects were 30.1 ± 10.3 years (range; 18 to 49 years), 9.5 ± 7.6 days (median; 4.5 and range; 2-39 days) 13.5 ± 2.7 (range; 6 -15) respectively. The hematological, biochemical and radiological parameters were normal in all patients. The nature of OPC was unknown in 5, Dichlorovas in 5, Dimethoate, Phorate, Monocrotophos and Propenofos in one each. Ten patients received only atropine as treatment and remaining 4 received atropine with pralidoxime (2-pyridine aldoxime methyl chloride). None of the patients developed intermediate syndrome.

Serum TSH at baseline though within normal range (0.7 ± 0.5) was lower at the time of admission compared to TSH at 3 months of follow up (2.9 ± 2.1) (*P* = 0.02). The levels of T_3 and T_4 did not differ significantly at baseline from that at 3 months. One patient developed new onset hypothyroidism with very low T_4 (3.0 μg/dl) and elevated TSH value of (6.7 uIU/ml) [Figure 2a, b and c]. His antithyroid peroxidase antibody was negative.

There was no significant difference between serum ACTH at admission to that at recovery and at 3 months follow-up [Table 1]. The levels of serum cortisol were significantly higher at baseline compared to that at 3 months (*P* = 0.004). At baseline 11 out of 14 patients had supraphysiological values of cortisol and 4 patients had sub-normal cortisol values. At discharge only 3 patients had sub normal values of cortisol which recovered at 3 months of follow up. At this juncture 5 patients had new onset sub normal cortisol values [Figure 3a and b]. However, all of them had normal cortisol response to IIH.
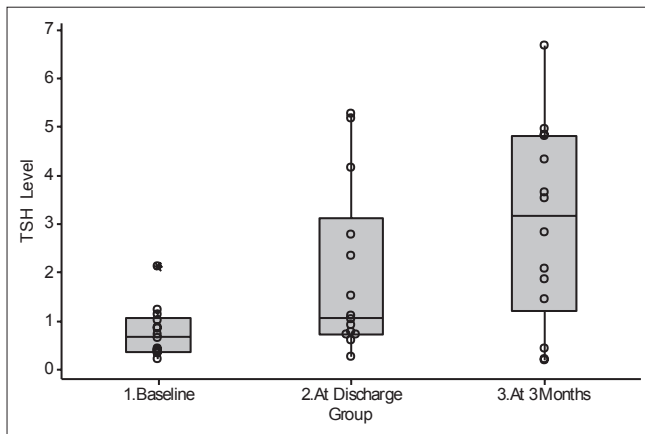

Figure 2a: The figure displays the distribution of TSH values at baseline (admission), at discharge and three months of follow-up
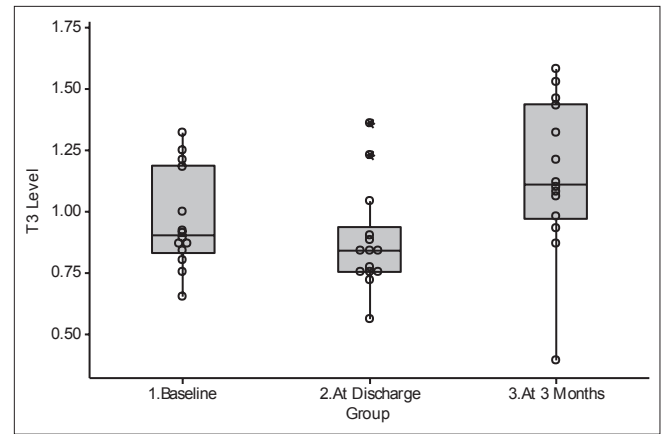

Figure 2b: The figure displays the distribution of T3 values at baseline (admission), at discharge and three months of follow-up
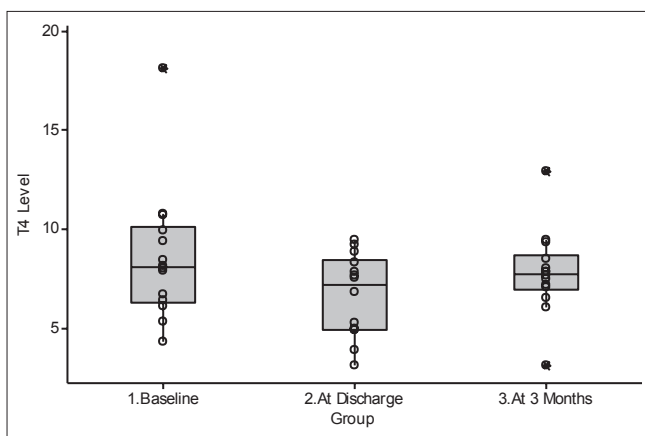

Figure 2c: The figure displays the distribution of T4 values at baseline (admission), at discharge and three months of follow-up
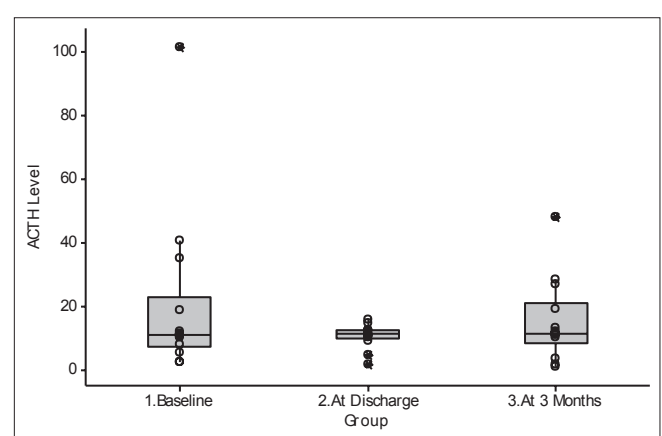

Figure 3a: The figure displaces the distribution of ACTH values at baseline (admission), at discharge and three months of follow-up

Table 1: Hormonal levels of patients at baseline (admission), at discharge and at three months after exposure to organophosphrous compound

Parameters (normal range)	Mean (±S.D) Median (IQ range)		
	Baseline	**At discharge**	**At 3 months**
TSH	0.7±0.50.6	1.9±1.7	2.9±2.1*
(0.27-4.2 uIU/ml)	(0.3-0.9)	1.1 (0.7-2.6)	2.8 (1.4-4.8)
T3	0.9±0.2	0.8±0.2	1.1±0.3
(0.8-2.0 ng/ml)	0.8 (0.84-1.1)	0.8 (0.7-0.8)	1.1 (0.9-1.32)
T4	8.5±3.3	6.6±2.0	7.7±2.2
(4.8-12.7 µg/ml)	8.0 (6.4-9.7)	7.1 (4.9-8.1)	7.6 (7.0-8.4)
ACTH	27.6±16.3	5.2±3.9#	15.0±11.2
(5-60 pg/ml (8 am))	3.8 (1- 16.7)	1 (1-3.9)	1.5 (1-19.0)
Cortisol	937.3±437.2	685.9±336.2	477.4±264.7*
(171-536 nmol/ml (AM)) (64-327 nmol/ml (PM))	939.4 (615.2-1245)	678.4 (542.0-855.2)	370.5 (239-682.2)
LH	4.5±4.2	4.4±3.8	5.1±2.4
(F; 2.4-12.6 mIU/ml) (M; 1.7-8.6 mIU/ml)	3.4 (0.6-8.3)	4.3 (0.4-6.6)	5.8 (4.3-6.9)
FSH	5.3±3.1	4.5±3.3	4.8±2.1
(F, 3.5-12.5 mIU/ml) (M, 1.5-12.4 mIU/ml)	4.9 (3.4-7.4)	4.2 (2.4-6.08)	4.0 (3.1-5.3)
PRL	17.5±13.7	13.2±4.4	9.9±3.0 *
(F,4.79-23.3 ng/ml) (M,4.0-15.2 ng/ml)	12.7 (8.5-18.2)	12.9 (10.4-15.3)	8.5 (8.1-11.3)
Testosterone	10.5±11.7	15.3±12.1	20.4±14.4
(9.9-27.8 nmol/ml)	6.1 (2.3-13.7)	13.3 (6.5-19.4)	21.5 (9.6-27.6)
Estradiol	48.2±37.8	48.6±17.3	153.4±77.6
(12.5-166 pg/ml)	48.0 (20.7-81.4)	43.8 (43.4-44.4)	* 186.5 (122.8-205.1)
DHEA-S	276.8±210.2	374.2±207.9	399.9±377.8
(F,57.5-395.3 µg/dl) (M,103.9-467.5 µg/dl)	296.1 (62.4-405.7)	349.6 (263.2-418.3)	310.6 (97.0-448.4)
GH	2.50±0.5	3.18±0.38	1.8±0.14
(0.005-5 ng/ml)	2.3	2.8	1.4
IGF-1	161.0±106.0	140.5±73.4	223.9±96.4**
(150-350 ng/ml for adults after puberty)	143.3 (94.6-168.1)	111.9 (90.0-190.5)	235.4 (159.9-285.0)

M: Male, F: Female, IQ: Inter quartile, T3: Triiodothyronine, T4: Thyroxine, ACTH: Adenocorticotrphic hormone, PRL: Prolactin, DHEAS: Dehydroepiandro sterone sulphate, LH: Luteinizing hormone, FSH: Follicular stimulating hormone, IGF-1: Insulin like Growth Factor-1, GH: Growth hormone. *denotes the statistical significant difference between baseline and at 3 months. #denotes the statistical significant difference between baseline and at discharge. **denotes the statistical significant difference at discharge and at 3 months. $P<0.05$ considered as statistical significant
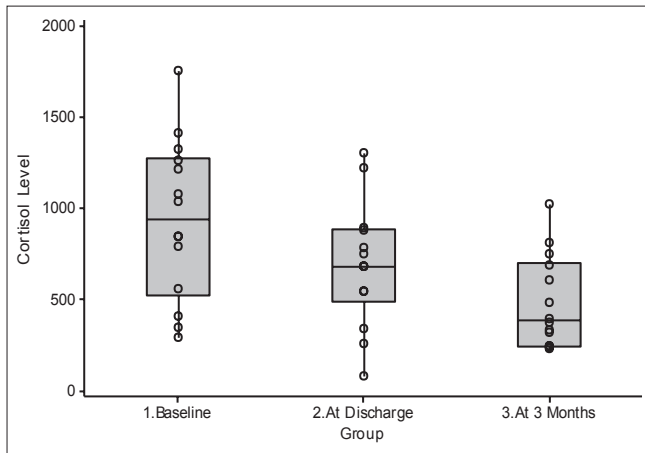

Figure 3b: The figure displays the distribution of cortisol values at baseline (admission), at discharge and three months of follow-up
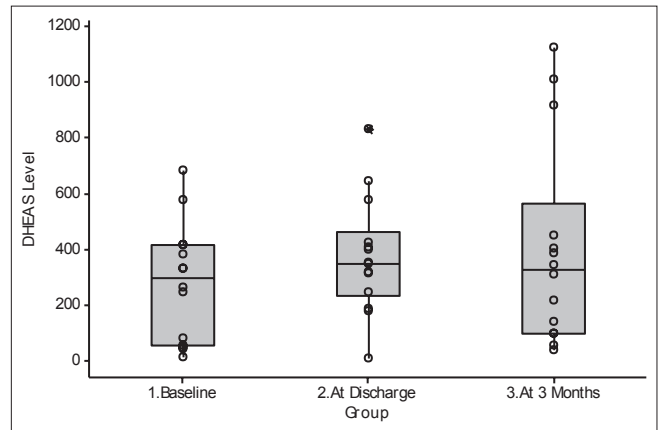

Figure 3c: The figure displays the distribution of DHEAS values at baseline (admission), at discharge and three months of follow-up

There was no significant difference in mean serum DHEA-S at recovery from acute intoxication after recovery and at 3 months follow up. However, DHEA-S was low at admission in 5 patients together with high cortisol but 2 had normal ACTH. At 3 month follow up one patient continued to have low DHEA-S with high cortisol and low ACTH while another had low DHEA-S with low-normal

cortisol and low ACTH [Figure 3c]. There was an inverse relationship between the serum cortisol and DHEA-S.

Serum PRL at baseline was higher than that at 3 months ($P = 0.04$). The serum LH and FSH values were not significant at baseline and on follow up [Figure 4]. Serum testosterone level was low in 6 out of 8 male patients at admission which normalised in three of them at discharge.

With 3 months of follow up one among the 6 continued to have low testosterone and another patient had new onset testosterone deficiency [Figure 5a-c]. Both these patients had normal LH and FSH inappropriate to the level of testosterone. In female patients there was no significant difference in serum 17 β-estradiol at baseline and at discharge; however, it was higher at 3 months. The serum 17 β-estradiol was low in 1 out of 6 women at admission which normalised at discharge. Four women at 3 months follow up had above normal 17 β-estradiol and all of them were associated with normal FSH and normal to sub-normal LH levels [Figure 5d].

There was no significant difference in the levels of IGF-1 at baseline and at 3 months follow up. However, there was statistically significant difference between IGF-1 levels at discharge and at 3 months ($P = 0.01$). Seven patients had low IGF-1 levels at baseline which recovered in 4 patients with 3 other patients developing new onset deficiency [Figure 6]. All of them had normal growth hormone levels at baseline. At follow up, 1 patient continued to have low level of IGF-1 since admission, while others recovered, and one patient who developed IGF-1 deficiency at discharge continued

to remain so at 3 month of follow up. Both of them failed to have inadequate GH reserve on IIH and MRI did not reveal any abnormality (data not shown in the box plot).

At baseline the mean random plasma glucose was 138.2 ± 43.5 mg/dl. Out of 14 patients, only one had transient hyperglycaemia with blood glucose more 200 mg/dl which normalised within 8 h of hospitalization and remained normal throughout the study period. None of patients developed hypoglycaemia.

Overall 7 patients out of 14 had nine hormonal deficiencies at the end of the study, 4 having sub normal cortisol, two each had testosterone and growth hormone deficiency and only one had T_4 deficiency at 3 months follow up.

There was a significant positive co-relation between cholinesterase with baseline IGF-1 ($r = 0.83$, $P < 0.001$), TSH ($r = 0.69$, $P = 0.02$), ACTH ($r = 0.74$, $P = 0.009$). There was no relationship between levels of acetyl cholinesterase and other hormones at baseline, discharge or at 3 months of follow up. The duration of hospital stay, atropine
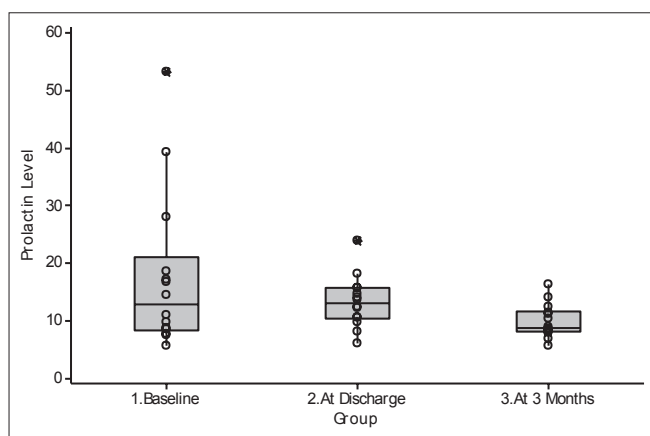

Figure 4: The figure displays the distribution of prolactin values at baseline (admission), at discharge and three months of follow-up
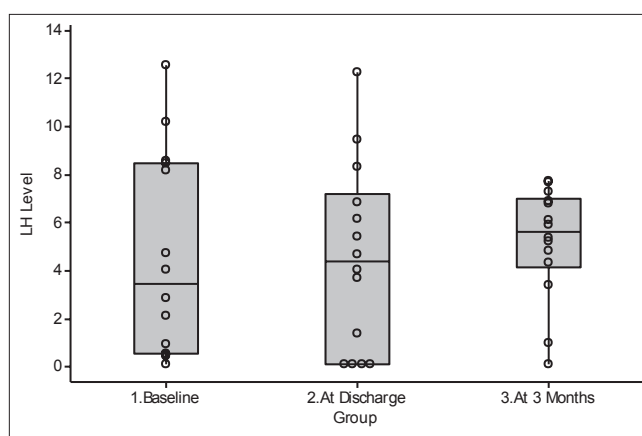

Figure 5a: The figure displays the distribution of LH values at baseline (admission), at discharge and three months of follow-up
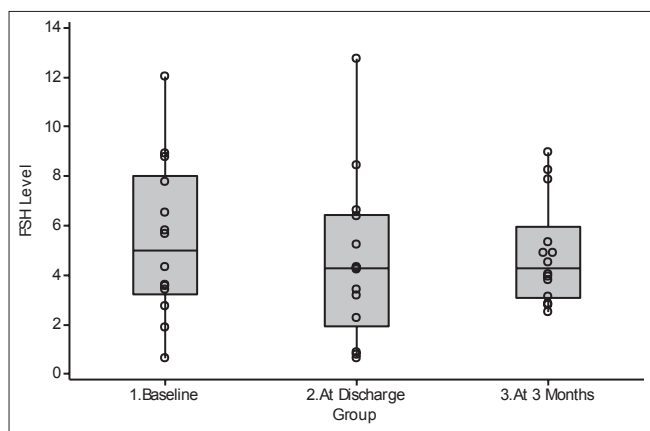

Figure 5b: The figure displays the distribution of FSH values at baseline (admission), at discharge and three months of follow-up
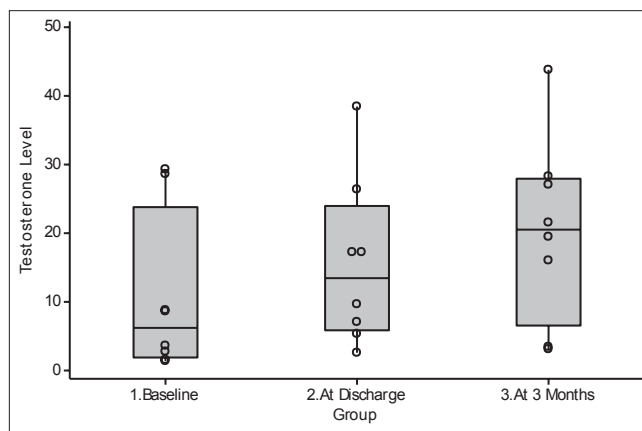

Figure 5c: The figure displays the distribution of Testosterone values at baseline (admission), at discharge and three months of follow-up
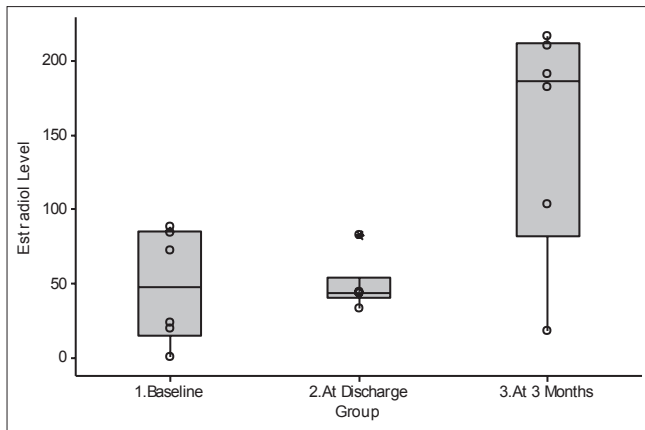
Figure 5d: The figure displays the distribution of Estradiiol values at baseline (admission), at discharge and three months of follow-up

Figure 6: The figure displays the distribution of IGF-1 values at baseline (admission), at discharge and three months of follow-up

and pralidoxime therapy did not influence the hormonal outcome.

DISCUSSION

Acute OPC poisoning produces sick euhormonal syndrome like any other acute stress. The TSH, ACTH and IGF-1 deficiencies at the time of presentation correlated with levels of cholinesterase. A small subset of patients had varying level of hormonal insufficiency either at diagnosis, recovery or at 3 months of follow up.

The mean serum T_3 and TSH were low-normal which were normalized at the time of discharge and at three months follow up. This pattern of altered thyroid function is similar to those observed during critical illness which is characterized by low T_3, low or low-normal T_4 and normal to low normal TSH.[9] In our study, the mean serum T_4 remained normal throughout the study period. The studies by Smallridge *et al.*,[12] and Güven *et al.*,[13] noted similar findings in thyroid function test. The variation in thyroid function during critical illness may depend on the severity of illness, duration of illness, levels of cytokines and age of the patient. In addition to the cytokine mediated suppression of TSH in critical illness, it is postulated that cholinergically mediated rise in somatostatin tone may also contribute to the suppression of TSH in patients with OPC poisoning. Furthermore, the nature of OPC may also affect the circulating thyroid hormone. Ultrastructural changes in thyroid tissue has been demonstrated in a study on rats with acute methamidophos poisoning suggesting that certain OPC may have direct deleterious effect on thyroid.[14] In our study, one patient had biochemical profile suggestive of hypothyroidism at admission and the T_4 decreased further with rise in TSH at 3 months of follow up. The TPO was negative and he had normal urinary iodine and no other identifiable cause of hypothyroidism.
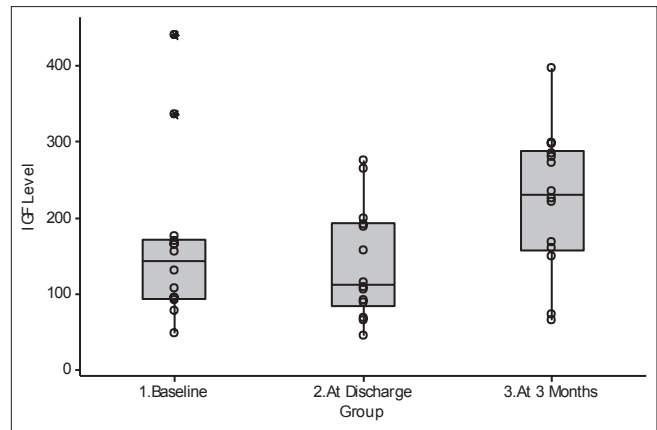
In the present study, the mean ACTH and cortisol were high or high normal at baseline that normalised at three months of follow up. The initial rise in glucocorticoid and ACTH in patients might have resulted from stress, cholinergically mediated effect on CRH and or ACTH secretion as none had cortisol deficiency at 3 months follow up.[10,11,19]

There was an inverse correlation between serum cortisol and dehydroepiandrosterone-sulfate (DHEAs) in our study. At three months of follow up, out of 5 patients who had poor glucocorticoid reserve three had low DHEAS and ACTH suggesting pituitary injury. Other two patients had normal DHEA sulphate and two patients developed new onset DHEA sulphate deficiency with normal ACTH and plasma cortisol indicating subtle adrenal insult. Teleologically this is beneficial for the survival and this can be explained by ACTH stimulated preferential rise in 11-deoxycortisol which acts as a precursor for cortisol synthesis. The alteration of DHEA-cortisol ratio may be defensive rather than mal-adaptive.[20,21]

Circulating PRL was normal at baseline and remained so at follow up in all except three who had ingested dichlorvos. The previous studies on PRL following OPC poisoning had conflicting results. This could be explained by action of acetyl choline on nicotinic receptor on the dopaminergic neurons in arcuate nucleus on lactotroph in pituitary and variable stress response which could be OPC specific or consequent to longer duration of stress.[22-25] We propose that dichlorvos may have direct action on dopamine pathway or hypothalamic neurons causing elevation of PRL levels.

There was no difference in serum LH and FSH at baseline and at follow-up. The study by Satar and colleagues has reported similar findings.[26] The mean testosterone was

marginally increased at 3 months follow up compared to that at admission. When individual patients were analysed two patients had low testosterone on 3 month follow up with normal LH and FSH. One of them consumed dichlorvas and in others the nature of OPC was not known.

Seven patients (50%) had low IGF-1 with normal GH at admission. At discharge, 6 out of 14 (42.8%) had low IGF-1, two of them continued to have persistently low IGF-1 on repeat evaluation at 3 months follow up. These two patients also had non-stimulable GH on IIH. The growth hormone IGF-1 axis normalized in all other patients. During acute stress, IGF-1 and IGFBP levels decreases despite rise in GH.[9] This acute phase response to stress, representing a peripheral resistance to GH is mediated by cytokines like tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6.[9] Acute rise in Ach in the hypothalamic neurons leads to increase in growth hormone (GH). This forms the pharmacological basis for the GH stimulation test using pyridostigmine to assess the GH reserve in GH deficiency children.[7,8] The deficiency on long term follow up could be due to direct pituitary injury in these two patients. Both of them ingested dichlorvas.

OPCs induced abnormalities in glucose homeostasis is a well known entity.[27] It is usually seen with malathion and diazinon. Hyperglycemia serves a useful tool to understand the etiology of OPC poisoning.[28] High plasma glucose (>200 mg/dl) was seen in only one of our patients at the time of admission which normalized within 6 h.

Mean ACTH, TSH and IGF-1 were positively correlated with acetyl cholinesterase which predicts the severity of poisoning. As the cholinesterase level recovers later, there is lack of co-relation on follow-up. There was no correlation with atrophine treatment, duration of hospital stay and hormonal insufficiencies.

The probable mechanisms of OPC induced delayed hormonal consequences as seen in some of our patients could be explained by complex extensive bio-transformation involving several metabolic systems with simultaneous oxidative injury at number of points utilizing cytochrome p-450.[29] Human beings have more efficient hydrolytic enzymes than insects thereby meaning more efficient detoxification process. There could be a lot of inter-individual variation in the detoxification process, dose of OPC and nature of OPC.

The strength of the current study is prospective nature, evaluating pituitary target gland hormones at admission, on recovery and at 3 months. Furthermore, this is the first study to show that there could be differential effect of OPC on pituitary-target gland axis. The limitations of the study are small number of recruited patients, undefined nature of OPCs in most, no comparative or control group and relatively "early after" assessment of hormonal parameters.

We conclude, initial hormonal changes in acute OPC poisoning are similar to sick euhormonal syndrome. These abnormalities disappear in most at recovery and in a small but significant proportion of patients may have hormonal insufficiency.

Acknowledgment

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