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Comparison between Creatinine phosphokinase and Serum Cholinesterase activity as indicator of prognosis in patients of organophosphorus poisoning.

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Abstract:

Background: Organophosphate poisoning leads to considerable mortality and morbidity across the world especially in developing countries. Extensive domestic and industrial use and availability of pesticides increases the chances of poisoning and long-term health effects.Our study aims tofind out enzyme activity like Serum creatinine phosphokinase (CPK) and Serum cholinesterase in organophosphorus poisoning, compare the enzymes as prognostic indicator and provide recommendations.

Methods and Results: This prospective, observationalstudy was conducted in the Department of General Medicine at M.K.C.G Medical college hospital, Berhampur during 2020 to 2021. Data was collected from 100 patients and their attendants; blood samples were investigated and data was analysed.Majority (60%) were females and 89% were suicidal. Median (IQR) age was 29 years and mean (SD) 32.8 (12.7) years. Familial issues (41%) were most common reason. Methyl parathion (40%) was the most common organo-phosphorous used, followed by chlorpyrifos (24%).Patients had pin point pupil(25%), depressed mental status (36%), secretions (74%), fasciculations (15%), respiratory failure (25%) and had intermediate syndrome (10%), bradycardia (25%), tachycardia (10%). Mild, moderate and severe secretions in 15%, 39% and 20% respectively. The median serum CPK levels decreased over from Day 1 (232) to Day 7 (134).The median serum Cholinesterase levels increased from Day 1 (2000.5) to Day 7 (4110). The mortality rate was 25%.Non-Survivors had significantly higher serum CPK levels than the survivors at Day 1as well as at Day 7 (1 week) ($p<0.05$) andlower serum cholinesterase levels

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than the survivors at Day 1(admission) as well as at Day 7 (1 week) ($p<0.05$).Survivors had significantly lower CPK levels and higher Serum cholinesterase levels at Day 7 than Day 1 of admission ($p<0.05$). Non-Survivors had both significantly higher Serum CPK levels and Serum cholinesterase at Day 7 than Day 1 of admission ($p<0.05$). Serum CPK had better predictive value for deaths than cholinesterase. A significant negative correlation between the serum CPK and cholinesterase levels at the admission (r-0.204, $p=0.041$). Non-survivors had significantly higher dose of atropine, pralidoxime, number of days in ventilator, ICU and overall hospital stay than the survivors ($p<0.05$).

Keywords: CPK; poisoning; pin point pupil; serum cholinesterase;suicidal

Introduction:

Organophosphate poisoning leads to considerable mortality and morbidity across the world especially in developing, low and low middle-income countries, that it is being held as public health problem.[(1)] The widespread use of the OP compounds as insecticide to increase the productivity of the crops, had made the accessibility and availability of them at ease in the nuke, corners and remote areas of India. This increased and easy availability coupled with absence of awareness regarding the outcomes of the OP poisoning has resulted in rampant usage.[(2)] Extensive domestic and industrial use and availability of pesticides in India, which is a developing country increases the chances of poisoning and long-term health effects.

In India, the poisoning by using the agents that are available at the household levels such as the carbamate substrates, organophophosphates, pyrethrinoids etc., has been found to the most common modality.[(13)] It has been estimated by the NCBI (National Crime Bureau of India) that among the suicidal poisoning cases reported in 2006 and 2007, 19% of the suicides were because of the pesticide consumption.[(14)]The number of people exposed to OPs has been calculated to be around 3 million across the world, which results in 3, 00,000 deaths thus transferring to a whopping 10% mortality rate. Global mortality rates with OP containing pesticides have been reported to be in that range of 2 to 25%. Malathion, trichlorfon and compounds like fenitrothion have been largely associated with mortality, among the OP poisonings.

It is reported that the OP poisoning causes around 3 lakh deaths in the region of Asia alone.[(3)]The outcomes of the OP poisoning in terms of morbidity and mortality have been interest of research across the world, especially in the developing countries owing to unequal burden. Clinical method of diagnosis is the first and important modality used for acute as well as chronic cases. Keeping OPC poisoning as one of the differential diagnoses, the same can be confirmed by atropine test. Gold standard is the measure of the RBC AChE activity, but it is costly and time consuming, which may impact the timely treatment of the OPC poisoning while the serum cholinesterase is utilized for its ease of quantification and the fast rate of decline in the blood. Additionally, other parameters such as the creatine phosphokinase has been widely evaluated for

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its supportive role in assessing the severity, outcome and prognosis of the OPC poison patients, which needs further evaluation in Indian settings.

Aims and objectives:

- To find out the different enzyme activity like Serum CPK and Serum cholinesterase in organophosphorus poisoning.
- To compare the enzymes as prognostic indicator
- To provide recommendations on the basis of the study findings

Materials and methods:

Study design: Hospital based, Prospective, observational, Study.

Study area:The present study was conducted in the Department of General Medicine at M.K.C.G Medical college hospital, Berhampurduring 2020and 2021.

Study population:

Inclusion Criteria:All patients aged more than 14 years and admitted to M.K.C.G Medical college hospital, Berhampur who had consumed organophosphorus compounds within 24 hours.

Exclusion Criteria: Patients with following conditions were excluded:

- Co-morbidities like myopathy, CKD, epilepsy, myocarditis, myocardial infarction, autoimmune disease, malignancies, pregnancy
- Trauma or received intramuscular injection and cardiopulmonary resuscitation recently
- Carbamate poisoning
- On prior medications like statins, fibrates, aspirin, anti-coagulants, furosemide and dexamethasone
- Alcoholics
- Succumbed immediately after admission
- Referred to other hospitals

Patients with co-morbidities like myopathy, CKD, epilepsy, myocarditis, myocardial infarction, autoimmune disease, malignancies, pregnancy, trauma, cardiopulmonary resuscitation recently, prior medications like statins, fibrates, aspirin, anti-coagulants, furosemide and dexamethasone; Carbamate poisoning, alcoholics also those who succumbed immediately after admission or referred to other hospitals.

Sample Size: 100 participants were taken as per inclusion and exclusion criteria.

Methodology: All eligible patients who are admitted in the Departments of Emergency and OPD, M.K.C.G Medical college hospital, Berhampur were included in the study. Once an organophosphorus poisoning patient is admitted, Data was collected from the patient and attendant, their blood samples were sent for the biochemical investigations at the hospital laboratory. The patient's serum cholinesterase and creatinine phosphokinase values were assessed at Days 1,2,3,4 and 7. All patients were followed up till 7 days for the outcomes in terms of survived or death. The duration of hospital stays, ICU stay as well as the atropine and pralidoxime requirement was noted.

Statistical Analysis

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Data was entered in MS Excel. Statistical analysis was done using SPSS v26.0. Categorical variables are presented in frequency and percentage (%). Chi-square test and Fishers exact test were used to test the significance of association between categorical variables. The continuous variables were found to be not normally distributed, by Kolmogorov-Smirnov test ($p<0.05$). Median and IQR was calculated for the continuous variables. Mann-Whitney test was used to significance in difference in the serum enzyme levels between the survivors and non-survivors. Wilcoxon signed rank test was used to test the significance in difference of the serum enzyme levels between day 1 and Day 7. Spearman correlation coefficient was calculated between the serum cholinesterase and creatinine phosphokinase at day 1. Predictive values of outcomes by the admission level CPK and cholinesterase was calculated in terms of sensitivity, specificity, positive predictive value and negative predictive value. A p value of <0.05 was considered statistically significant. Appropriate box plots, pie-charts, bar graphs & scatter plots were used to depict the results

The study was carried out after getting ethical clearance from the Institutional Ethics Committee, M.K.C.G Medical college hospital, Berhampur. Informed written consent was obtained from the patient after explaining the benefits and harm related to the study.

Results:

Majority of the study participants were females (60%) and had intermittent exposure (90%). Median (IQR) age was 29 (24,42) years. Mean (SD) age was 32.8 (12.7) years. Familial issues (41%) were most common reason for exposure to the OP poisoning followed by frustration in love affair (18%).[Figure 1]Maximum cases (89%)were of suicidal poisoning[Table 1].Methyl parathion (40%) was the most common OP agent used by the patients, followed by chlorpyrifos (24%), monocrotophos (16%) and fenthion (10%)[Figure 2]. Consuming the OP compound alone was the most common mode of ingestion (55%), followed by mixing it with water (38%), alcohol (5%)and milk (3%).

In our study the patients had pin point pupil(25%), depressed mental status (36%),secretions (74%), fasciculations (15%), respiratory failure (25%) and had intermediate syndrome (10%).25% of the patients had bradycardia while 10% had tachycardia. In our study, mild, moderate and severe secretions were present in 15%, 39% and 20% of the patients, respectively.Table 2 shows that the median serum CPK levels decreased over the period of time from Day 1 (232) to Day 7 (134).The median serum Cholinesterase levels increased over the period of time from Day 1 (2000.5) to Day 7 (4110). In the study participants, 85%, 80%, 65%, 60% and 34% had abnormal cholinesterase levels at Days 1, 2, 3, 4 & 7, respectively.Also, 74%, 69%, 69%, 40% and 25% had abnormal CPK levels at Days 1, 2, 3, 4 & 7, respectively[Table 3]. The mortality rate was 25% among our patients.

Mann-Whitney test was used to test the difference in the serum enzyme levels between the survivors and non-survivors. A p value of <0.05 is considered as statistically significant. Non-Survivors (Deaths) had significantly higher serum CPK levels than the survivors at Day 1(admission) as well as at Day 7 (1 week) ($p<0.05$) [Table 4].

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Non-Survivors (Deaths) had significantly lower serum cholinesterase levels than the survivors at Day 1(admission) as well as at Day 7 (1 week) ($p<0.05$) [Table 4].Wilcoxon signed rank test was used to test the significance in difference of the serum enzyme levels between day 1 and Day 7. Survivors had significantly lower CPK levels at Day 7 than Day 1 of admission ($p<0.05$) [Figure 3,4], alsothey had significantly higher Serum cholinesterase levels at Day 7 than Day 1 of admission ($p<0.05$)[Figure 5,6].Non-Survivors had significantly higher Serum CPK levels at Day 7 than Day 1 of admission ($p<0.05$). Non-Survivors had significantly higher Serum cholinesterase levels at Day 7 than Day 1 of admission ($p<0.05$).Serum CPK was found to have better predictive value for deaths in OP poisoning in our study than the cholinesterase [Table 5]. There is a significant negative correlation between the serum CPK and cholinesterase levels at the admission (r-0.204, $p=0.041$) [Figure 7]. Dose of atropine and pralidoxime were higher among the non-survivors than the survivors ($p<0.05$) [Table 6]. Non-survivors had significantly higher number of days in ventilator, ICU and overall hospital stay than the survivors ($p<0.05$) [Table 7].

Discussion:

Organophosphate compound poisonings are frequent in developing countries and is of significant concern, as the economically most productive age groups are majorly affected in such countries. The present study included 100 patients who consumed organophosphate compounds from Berhampur, Odisha.

Mortality rate was 25% in our study, in line with Cunha et al[(5)]who reported 28.9% in ICU settings but slightly higher than the rate reported by Dhanalakshmi et al (17%)[(6)] who conducted a similar study among children,and lower than the rate reported by Banday et al (33.3%).[(7)]

Mean (SD) age was 32.8 (12.7) years, similar to Kumar et al (Mean-31.5 years),[(8)] lower than one reported by Kang et al[(9)] (54.5 years),(44) and higher than Bhattacharya et al[(10)] (Mean-25.5 years) and Dhanalakshmi et al (Mean-12.6 years).[(6)] The difference might be due to the fact that study population only children in Dhanalakshmi et al,[(6)] while we included all patients above 14 years. Majority of the study participants were females (60%), similar to previous studies by Sen et al and Dhanalakshmi et al,[(6)] while males were reported as majority by other studies.[(7,8, 9, 10,11)]

Methyl parathion (40%) was the most common OP agent used by the patients, followed by chlorpyrifos (24%), while previous studies reported Chlorpyrifos,[(6,7,10)]dichlorvos,[(9)]methidathion,[(12)] and diethomate,[(7)] as the most common compound. The varied compounds may be due to the local factors.

Among the patients in our study, 25% had pin point pupil, 36% had depressed mental status, 74% had secretions, 15% had fasciculations, 25% had respiratory failure and 10% had intermediate syndrome. Vomiting was the most common symptom reported by a similar study by Bhattacharya et al (87.3%), while miosis and fasciculation was reported by 81% and 25.4%.[(10)] While 25% of our patients had bradycardia and 10% had tachycardia, Bhattacharya et al reported, contrastingly, 69.84% had bradycardia and 3.2% had tachycardia. Banday et al reported that 93.2% and 33.8% had miosis and salivation / lacrimation.[(7)] Complications such as respiratory paralysis and intermediate syndrome was reported by 15.9% and 7.9% by

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Bhattacharya et al,[10] which was different from ours. The differential proportion of symptom pattern and complications might be due to the differential compound which was found to be used in the two studies and the treatment facilities available at the two settings at different time points.

The median serum CPK levels decreased over the period of time from Day 1 (232) to Day 7 (134), which was in line with results reported by John et al.[13] Kumar et al reported a slightly different results wherein the CPK levels peaked at 48 hours and then fell by about 96 hours.[8] The serum levels of CPK reaches the peak either by 24 or 48 hours since the initiation of the muscle injury. The CPK levels then falls at a rate of 39%, on successive days, and the rate remains fairly constant.[15]

Among our study participants, 74%, 69%, 69%, 40% and 25% had abnormal CPK levels at Days 1, 2, 3, 4 & 7, respectively. Muscle injury is inevitable in the OP poisoning. It starts at the time of cholinergic crises and the injury severity is associated with the cholinergic crises severity.[13]Non-Survivors (Deaths) had significantly higher serum CPK levels than the survivors at Day 1(admission) as well as at Day 7 (1 week) ($p<0.05$). Our findings reverberate the results from other studies that had similar findings at end of week 1 by Dhanalakshmi et al,[6] and at Day 1 by Sen et al.[15] High CPK levels and low serum cholinesterase levels in the non-survivors and vice-versa in survivors adds evidence to the probable usefulness of the enzymes as prognostic marker for survival in OP poisoning.

In the present study, we found that the survivors had significantly lower CPK levels at Day 7 than Day 1 of admission ($p<0.05$) while the non-survivors had significantly higher Serum CPK levels at Day 7 than Day 1 of admission ($p<0.05$), which is exactly in line with the results reported by Dhanalakshmi et al.[6] This depicts a pattern of increase in cholinesterase levels and the decrease in the serum CPK levels, as the days progress from the OP poisoning administration, irrespective of the patient outcome. Bhattacharya et al reported that OP poison with mild severity had significant reduction in CPK levels at the end of 1 week than at admission, while the ones with increasing severity of poisoning showed no such significant change, which might have been owing to the complications the severe cases would have developed during the clinical course.[10] Hassan et al slightly varied from Bhattacharya in that OP poisoning with mild and moderate severity were deemed to have significantly reduced CPK levels at later days than at admission time, while no such variation was found in severe cases.[14] CPK values in recovering patients have been shown to fall over the period of time.[10]

The median serum Cholinesterase levels increased over the period of time from Day 1 (2000.5) to Day 7 (4110), similar to Sen et al[15] and Cunha et al.[5] Cunha et al concluded that the enzyme can be used for diagnosis as well as the monitoring the clinical course of the OP poisoning. It has also been reported that when serum Cholinesterase recovers over 10% of the normal, it correlated with good prognosis.[5] Among the study participants, 85%, 80%, 65%, 60% and 34% had abnormal cholinesterase levels at Days 1, 2, 3, 4 & 7, respectively.

Non-Survivors (Deaths) had significantly lower serum cholinesterase levels than the survivors at Day 1(admission) as well as at Day 7 (1 week) ($p<0.05$), in line with the findings from other

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studies at end of week 1.[6] Sen et al reported that day 1 serum cholinesterase,[15] while Kang et al and Yun et al found that initial serum cholinesterase levels were significantly higher in survivors than the non-survivors,[9,12] similar to our findings. Banday et al reported that serum pseudocholinesterase levels were significantly associated with the mortality.[7]Nouira et al, one of the earliest studies in this arena (1994),[16] reported that serum Cholinesterase measured within 24 hours of admission was not a significant predictor of severity of OP poisoning. This finding which is starkly different from the current literature might be due to differential methodology and the laboratory techniques used at different time periods.

Both Survivors as well as non-survivors were found to have significantly higher Serum cholinesterase levels at Day 7 than Day 1 of admission ($p<0.05$), in our study, which has also been reported by Sen et al in their study.[15] In a study among the sarin poisoning from Japan, cholinesterase has been found to return to normal in 3 months of time.[17]

Serum CPK was found to have better predictive value for deaths in OP poisoning in our study than the cholinesterase. Admission day Serum CPK was found to have 34.7% sensitivity and 33.8% negative predictive value, higher than that of the serum cholinesterase (Sensitivity- 20%, Negative predictive value- 29.4%). Sumathi et al found serum CPK to have diagnostic value towards the severity of OP poisoning,[18] while Hassan et al concluded that initial CPK values can diagnose acute OP poisoning.[14] Not just OP poisoning, serum CPK levels has shown to be a good prognostic marker for poisoning in general.[19,20]Thus the specificity of the enzyme with regards to specificity towards OP poisoning needs to be probed further in future studies.

In our study, we found a significant negative correlation between the serum CPK and cholinesterase levels at the admission ($r=-0.204$, $p=0.041$). This is in line with the findings of Dhana Lakshmi et al who also reported a negative correlation of higher magnitude ($r=-0.522$, $p=0.002$),(34) while Sumathi et showed no such correlation.[18] Hassan et al reported a significant negative correlation between butyryl cholinesterase and serum CPK,[14] while Bhattacharya et al found a negative correlation between erythrocyte cholinesterase and serum CPK in a similar age group of patients.[10]

Non-survivors had significantly higher number of days in ventilator (Median- 7 vs 0 days), ICU (Median- 10 vs 0 days),and overall hospital stay (Median- 10 vs 5 days), than the survivors ($p<0.05$), while Yun et al reported no such difference in the above parameters between the survivors and deceased OP poisoning patients.[3]

Conclusion:

The serum CPK levels decreased and serum cholinesterase levels increased over the period of time in the OP poisoning patients. Serum CPK level and serum cholinesterase are effective prognostic markers for predicting the outcome in the patients with OP poisoning. Serum CPK levels might be a better prognostic indicator in the acute OP poisoning for predicting the mortality of the patients. It is easily available and of low cost. As our study was prospective studytemporal association could be elicited and the biochemical parameters studied using standard laboratory techniques. The power of the study is limited because of lack of adequacy of sample size, the study has limited internal and external validity as non-random sampling was

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done in a single centre.Future, multi-centric studies with prompt calculation of sample size with adequate power must be conducted to confirm the findings of our study and to provide more evidence on recommending serum CPK as a reliable marker of prognosis in predicting the outcomes among the OP patients.

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Manner of poisoning	Frequency	Percentage
Suicidal	89	89.0
Homicidal	2	2.0
Accidental	9	9.0
Total	100	100.0

Table 1- Manner of poisoning

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Days	CPK levels		Cholinesterase levels	
	Median	IQR	Median	IQR
Day 1	232	154,413.5	2000.5	1234,3177.5
Day 2	211	157,499.5	2200	1880,3663.5
Day 3	184	113,512	3057.5	2450,4000
Day 4	150	103,590	3160.5	3000,4215.3
Day 7	134	86.3,706.8	4110	3147,5075.5

Table 2: Median CPK and Median Serum Cholinesterase levels

Category	Levels	CPK		Serum cholinesterase	
		Frequency	Percentage	Frequency	Percentage
Day 1 category	Normal	26	26.0	15	15.0
	Abnormal	74	74.0	85	85.0
Day 2 category	Normal	31	31.0	20	20.0
	Abnormal	69	69.0	80	80.0
Day 3 category	Normal	31	31.0	35	35.0
	Abnormal	69	69.0	65	65.0
Day 4 category	Normal	60	60.0	40	40.0
	Abnormal	40	40.0	60	60.0
Day 7 category	Normal	75	75.0	66	66.0
	Abnormal	25	25.0	34	34.0

Table 3: Serum CPK and creatinine phosphokinase levels as per day category

Enzyme Activity	Survivors		Non-Survivors		p value
Serum CPK	Median	IQR	Median	IQR	
At Day 1 (Admission)	223	154,247	558	550,679	<0.001
At Day 7 (1 week)	103	80,141	1084	1068,2041	<0.001
Serum Cholinesterase					
At Day 1 (Admission)	2001	1234,3555	2000	1122,2110	0.024
At Day 7 (1 week)	4112	3147,5687	3250	3110,4000	0.002

Table 4: Enzyme activity in Survivors and non-survivors

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	CPK	Cholinesterase
Sensitivity	34.7	20
Specificity	100	100
Positive Predictive Value	100	100
Negative Predictive Value	33.8	29.4

Table 5: Predictive values of outcomes by the admission level CPK and cholinesterase

Treatment	Survivors		Non-Survivors		p Value
	Median	IQR	Median	IQR	
Dose of Atropine	25	20,40	130	110,130	<0.001
Dose of Pralidoxime	4	3.5,4.5	5	5,5.5	<0.001

Table 6: Dose requirement of atropine and pralidoxime.

Days of treatment	Survivors		Non-Survivors		p Value
	Median	IQR	Median	IQR	
Number of days in ventilator	0	0,0	7	6,8	<0.001
Number of days in ICU	0	0,0	10	8,10	<0.001
Number of days in Hospital	5	4,6	10	10,11	<0.001

Table 7: Days of treatment and hospital stay.

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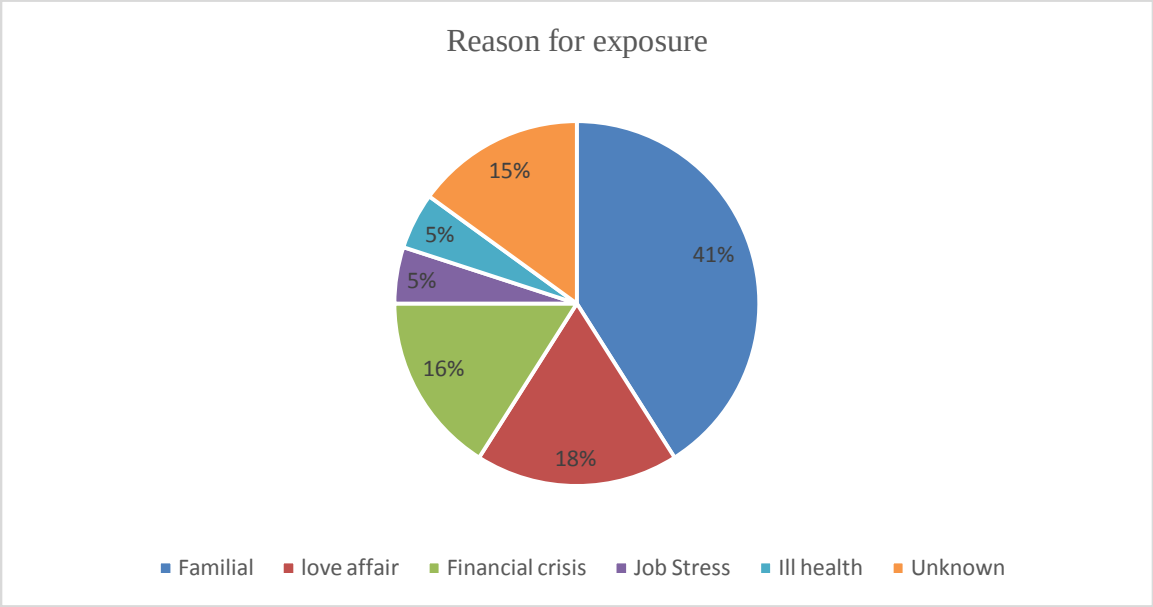

Figure 1: Reason for exposure.

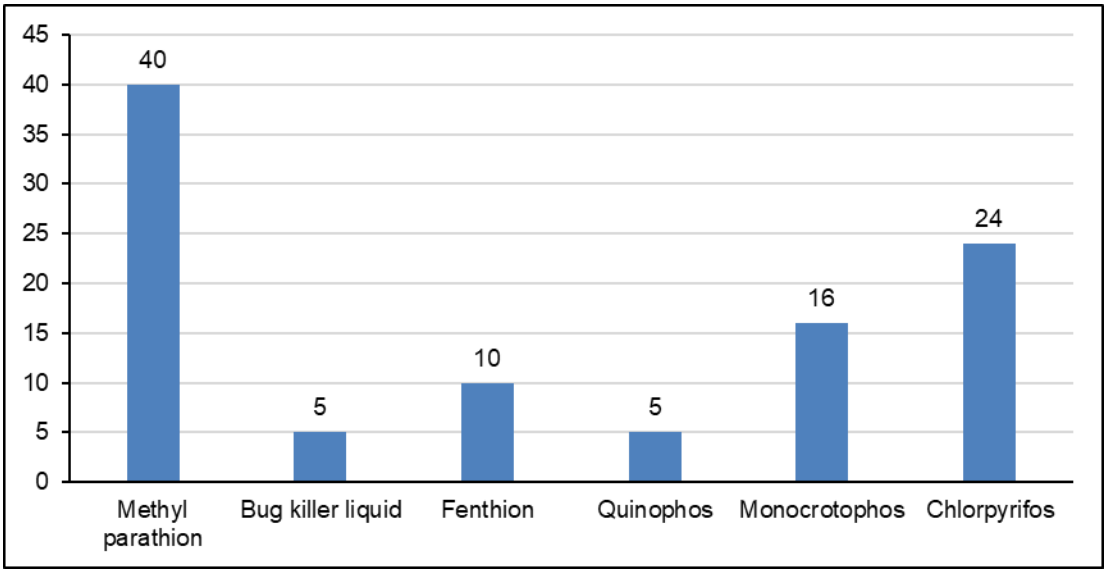

Figure 2: Type of compound exposed.

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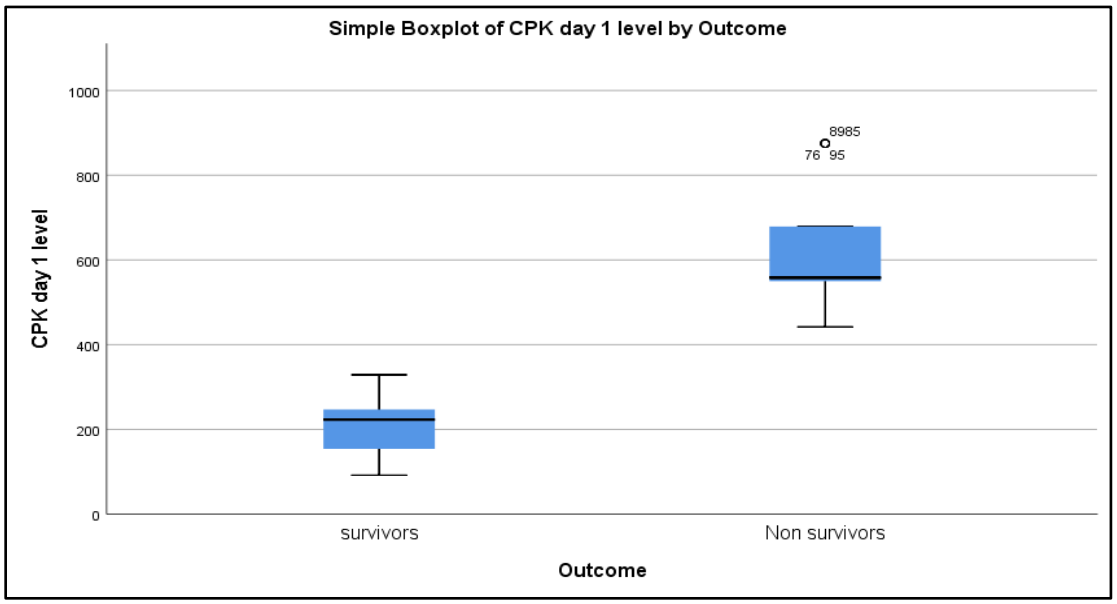

Figure 3: Simple Boxplot of CPK day 1 level by outcome

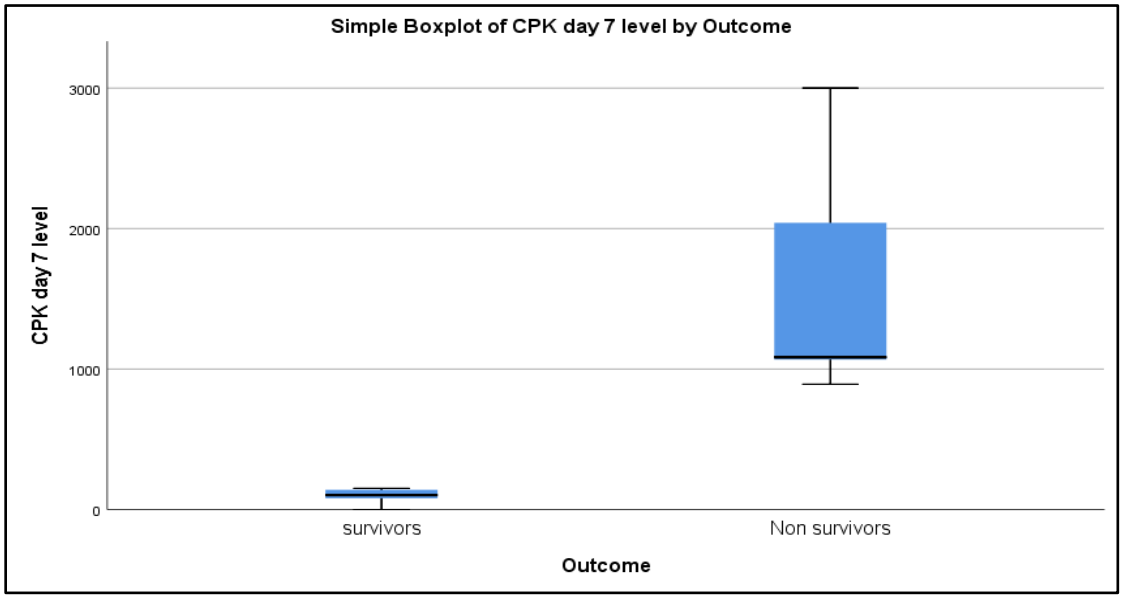

Figure 4: Simple Boxplot of CPK day 7 level by outcome

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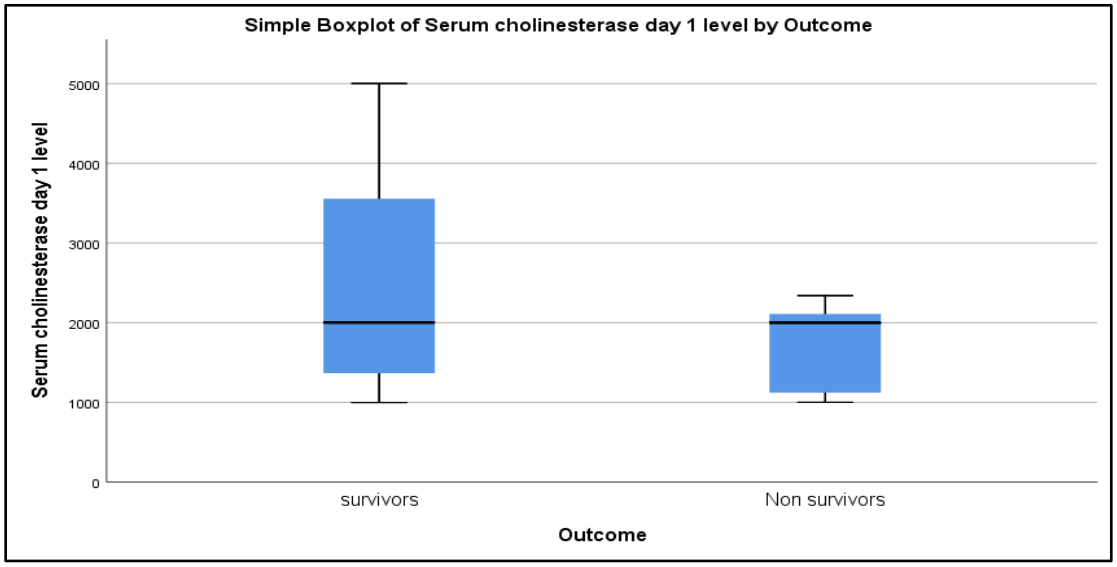

Figure 5: Simple Boxplot of serum cholinesterase day 1 level by outcome

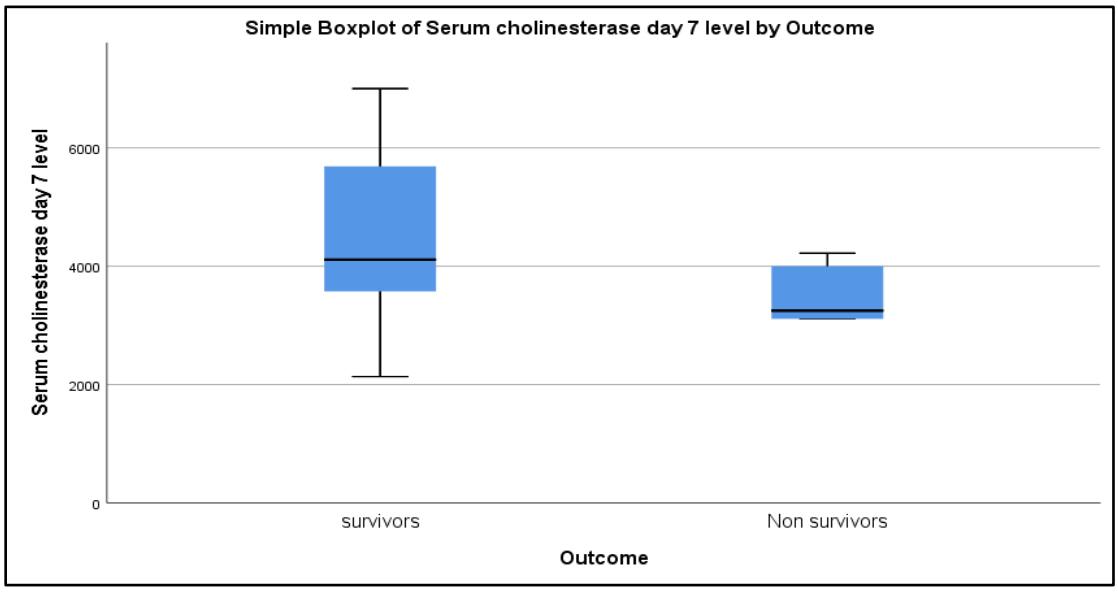

Figure 6: Simple Boxplot of serum cholinesterase day 1 level by outcome

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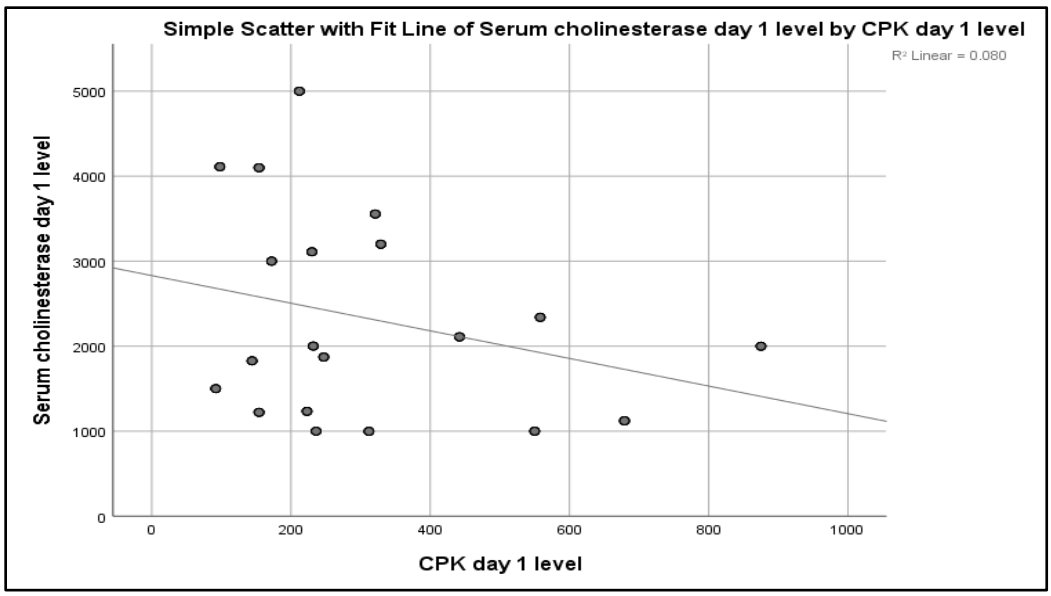

Figure 7: Simple scatter with Fit line of Serum Cholinesterase Day 1 level by CPK day 1 level.