Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: www.saspublishers.com

OPEN ACCESS

Clinical Biochemistry

Methods and their Reliability for the Determination of Serum Paraoxonase1 (PON1) Phenotypes in Acute Organophosphorus Compound Poisoning

Dr. Ananthi P[1], Dr. Cactus Lily Jeyaraj[2*]

[1]Assistant Professor, Department of Biochemistry, Govt. Villupuram Medical College, Villupuram, Tamilnadu, India
[2]Assistant Professor, Department of Biochemistry, Tirunelveli Medical College Tirunelveli- 627011 Tamilnadu, India

***Corresponding author:** Dr. Cactus Lily Jeyaraj
| **Received:** 22.02.2019 | **Accepted:** 02.03.2019 | **Published:** 13.03.2019
DOI: 10.36347/sjams.2019.v07i03.002

Abstract | **Original Research Article**

Acute organophosphorus poisoning is one of the medical emergencies in India. OPCs inhibit Cholinesterase enzyme and cause neuromuscular toxicity whereas OPCs are degraded by a polymorphic enzyme Paraoxonase1 (PON1). Hence PON1 phenotyping in OPC poisoned patients influences their management protocol. Phenotyping can be done by measuring the individual activity of Paraoxonase or Arylesterase or by taking the ratio of their activities. In the present study, we aimed to analyze these methods to assess their reliability by correlating them with cholinesterase activity which is needed for the effective usage of PON1 phenotyping in acute OPC poisoning. Serum Cholinesterase activity was estimated by the DGKC-kinetic method. Arylesterase and Paraoxonase activities of PON1 were estimated by Modified Zeller and Eckerson., *et al* methods respectively. SPSS software was used for statistical analysis. From this study, it was found that either the paraoxonase activity or the ratio can be used for PON1 phenotyping or to assess PON1 activity in patients with acute OPC poisoning since they had the correlation value of. +0.767 and +0.955. However, it can be concluded that the 'ratio calculation' which includes both the allozyme activities of paraoxonase1 is the more reliable method.

Keywords: Acute organophosphorus poisoning, PON1 phenotyping, Paraoxonase activity, Arylesterase activity, Serum Cholinesterase.

INTRODUCTION

'Poisoning' is a significant cause of ill health and mortality around the world. This may be accidental, suicidal or homicidal in mode. With respect to the drastic changes in agriculture for improving the cultivation, there is increasing in the usage of pesticides and insecticides worldwide. This productive development is also being reflected in the poisoning of humans especially with Organophosphorus compounds (OPC) all over the world [1, 2]. The reported proportion of admissions due to acute OPC poisoning in India is around 10-12% [3,4].

Organophosphorous compounds (OPC) cause neuromuscular toxicity with various grades due to the inhibition of enzymes namely Acetyl Cholinesterase and Neuropathy Target Esterase and also due to its induction of oxidative stress [5-7]. Currently, serum Cholinesterase (CHE) activity is used as a sensitive marker to monitor the disease progress and to grade the severity of toxicity. It is also being helpful to assess the response of the patient to the therapy, yet it could not be relied upon for the prediction of OPC level in blood and the extent of toxicity [8-10].

With a high concentration of OPC in the blood and tissues, there will be re-inhibition of CHE that may lead to failure of response even with a high dose of oxime therapy [10,11]. To improve the response of the disease to oximes, the active forms of OPC in blood and tissues must be at a low level. This depends on the dose and clearance of active OPCs from the body which directed the studies on the metabolism of OPCs.

The degradation of active OPCs and the reduction of oxidative stress are influenced by Paraoxonase1 (PON1) which is a polymorphic xenobiotic enzyme. The level of enzyme activity of PON1 shows individual variations [12,13]. The phenotypic differences or polymorphism in enzyme activities of PON1 are determined by its two allozymic activities namely Paraoxonase and Arylesterase which are dictated by the genome of PON1 at the coding region Q192R[13-15].

From the previous studies, it was proposed that the determination of the PON1 phenotype in an individual will help to identify their susceptibility to OPC toxicity and to modify their management protocol

in OPC poisoning[13,15,16]. There are different methods for serum PON1 phenotyping either by measuring only its individual allozyme activities or by calculating their ratio [17-19].

Hence we aimed to analyze these methods and their reliability for the determination of serum Paraoxonase1 (PON1) phenotypes in acute organophosphorus compound (OPC) poisoning. The objectives of this study were to estimate the activities of PON1 allozymes namely Paraoxonase and Arylesterase, to calculate the ratio of PON1 allozymes activities, to assess the correlation of the individual allozyme activities of PON1 and their ratio with serum cholinesterase activity and to evaluate the reliability of the methods in the determination of the phenotypes of the PON1 enzyme.

MATERIALS AND METHODS

The present analytical study was performed in the clinical Biochemistry laboratory of Govt. Stanley Hospital, Chennai over 6 months of duration. The blood samples and the clinical data were collected from 52 OPC poisoned patients, admitted in the Intensive Care Unit within a day of exposure. The study was conducted with the approval of the Ethics Committee and with the informed consent obtained from the participants of this study.

The Cholinesterase activity was estimated by the DGKC-kinetic method using Erba reagent kit. The Paraoxonase activity of PON1 was estimated using the substrate paraoxon by Eckerson *et al.* method and the Arylesterase activity of PON1 was estimated using phenylacetate by Modified Zeller's method [12,14]. The procedures for the above enzyme activities were standardized with the quality control sera (QC) prepared from serum samples of apparently healthy volunteers who attended the health checkup clinic. The intra and inter-assay variability were assessed with QC sera and the patients' sera.

The estimated data were statistically analyzed with the software SPSS-version 16 package. The values of standardization procedures were expressed as mean, standard deviation and coefficient of variation. The values of QC sera were plotted as Levey Jennings chart.

'Pearson coefficient of correlation' was used to analyze the level and strength of correlation between individual allozyme activities and their ratio with serum Cholinesterase activity. The 'p' value to assess the level of significance was taken as '0.05'. Statistically analyzed results are reported as Tables & Figures.

RESULTS

The standardization of the enzymatic assays was done by analyzing the stability of the substrates (Table-1.1) and the intra and inter assay variability of Paraoxonase and Arylesterase enzyme activities (Table 1.2a and 1.2b). The quality control done for enzyme activities of Cholinesterase, Paraoxonase, Arylesterase in pooled QC sera are shown in Table 2 & Figures1,2 and 3. The mean values of cholinesterase, paraoxonase and Arylesterase activities of the study population were 2325U/L, 218.39U/L and 85.13kU/L (Table 3). The correlation of cholinesterase activity was noted significantly positive with Paraoxonase activity +0.767 and with the ratio of PON1 allozyme activities +0.955 but the association of CHE with Arylesterase activity was weak and negative -0.239 (Table 4 & Fig. 4,5,6).

Table -1: Standardization of the test procedures

Table-1.1: **Non-enzymatic hydrolysis of the substrates**

Substrate	Mean	Standard Deviation(2 SD)
Paraoxon (U/L)	3.1	0.65
Phenylacetate (kU/L)	0.26	0.09

Table-1.2a: Intra assay variability

Measures	Serum Paraoxonase activity (U/L)	Serum Arylesterase activity (kU/L)
Mean	294.34	72.14
Standard Deviation(2 SD)	7.82	1.52
CV (%)	2.66	2.11

Table-1.2b: Inter assay variability

Measures	Serum Paraoxonase activity (U/L)	Serum Arylesterase activity (kU/L)
Mean	287.24	71.29
Standard Deviation(2SD)	9.36	1.96
CV (%)	3.26	2.75

Table-2: Enzyme activity values of quality control sera

Serum Enzyme activity	Mean	Standard Deviation(2 SD)
Cholinesterase (U/L)	7820	33.13
Paraoxonase (U/L)	197.87	4.68
Arylesterase (kU/L)	62.31	2.32

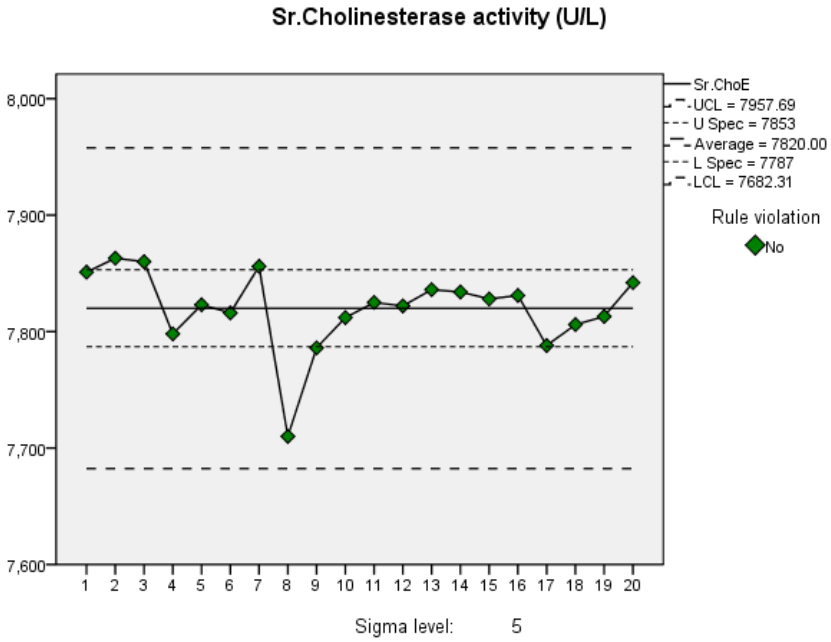

Fig-1: Quality control chart for serum Cholinesterase activity

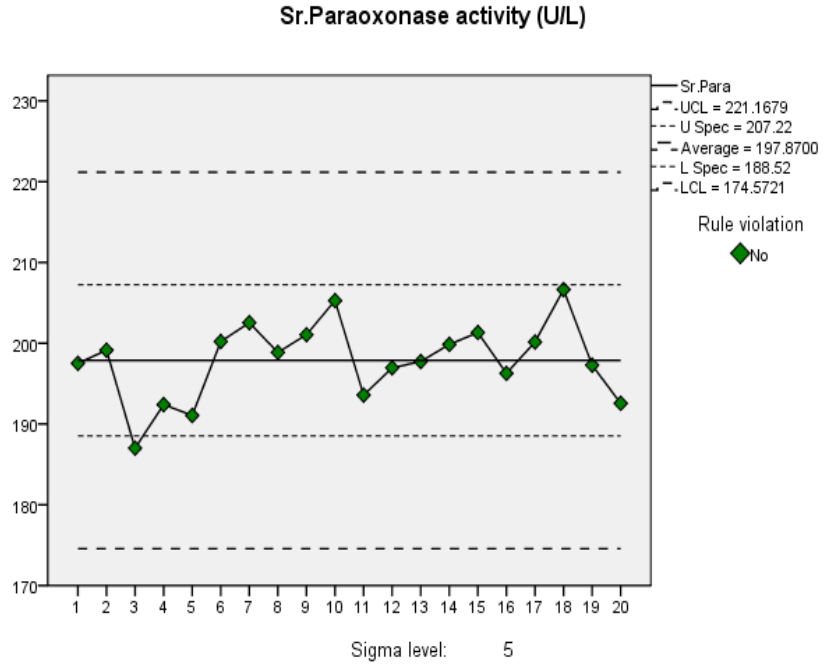

Fig-2: Quality control chart for serum Paraoxonase activity

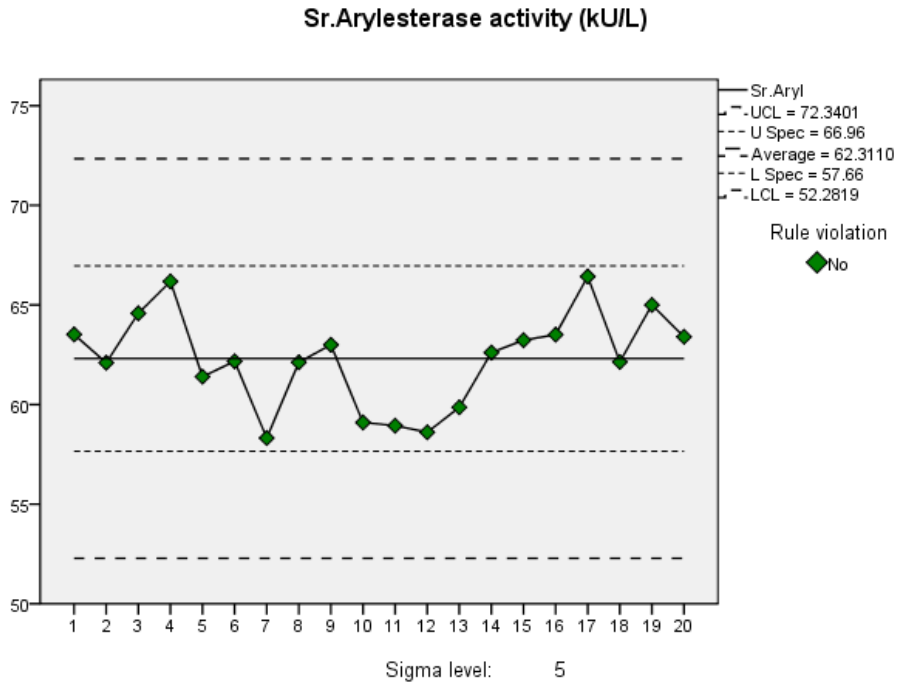

Fig-3: Quality control chart for serum Arylesterase activity

Note : Fig.1-3 are Levey Jennings Chart for serum Cholinesterase, Paraoxonase & Arylesterase activity. x-axis- Days; y-axis- mean, ±1SD, ±2SD values of enzyme activity.

Table-3: Enzyme activities of the study population

Serum Enzyme activity	Minimum	Maximum	Mean	Standard Deviation(2 SD)
Cholinesterase (U/L)	342	9840	2325	2027.19
Paraoxonase (U/L)	49.86	494.28	218.39	124.30
Arylesterase (kU/L)	41.73	191.35	85.13	30.19

Table-4: Association of serum Cholinesterase activity with the activities of Paraoxonase, Arylesterase & P/A ratio

Variables	Coefficient of Correlation (r)	Level of Significance (p)	Strength of Association	Interpretation
Sr.Cholinesterase vs Sr.Paraoxonsae	+0.767	< 0.0001	Strong association	Significant with positive correlation
Sr.Cholinesterase vs Sr.Arylesterase	-0.239	> 0.05	Weak association	Not Significant with negative correlation
Sr.Cholinesterase vs Sr.P/A ratio	+0.955	< 0.0001	Strong association	Significant with positive correlation

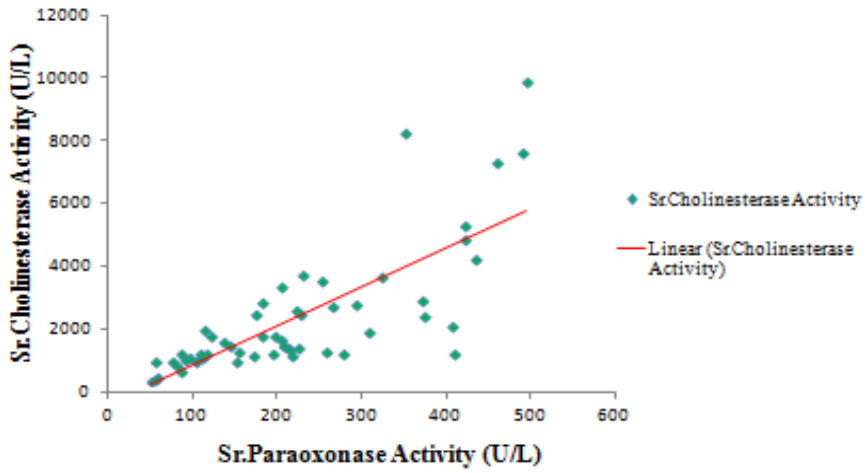

Fig-4: Serum cholinesterase vs paraoxonase activity

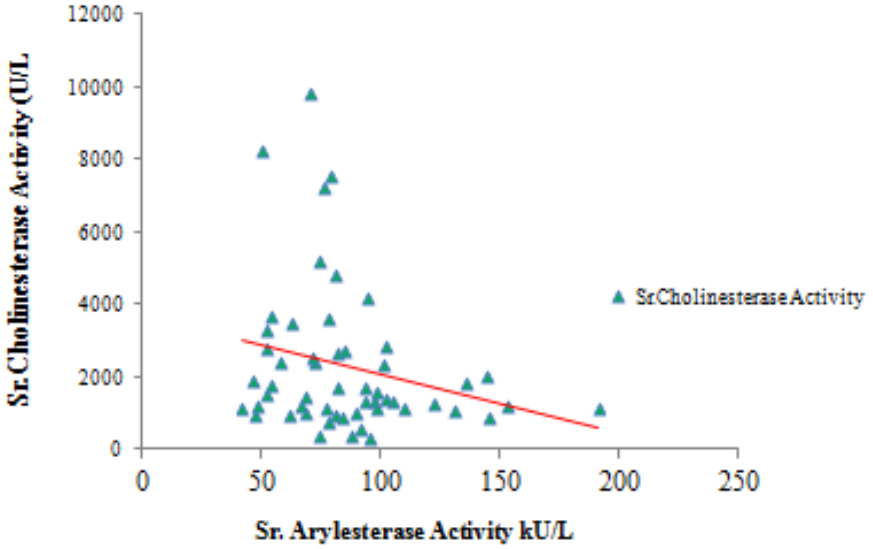

Fig-5: Serum cholinesterase vs arylesterase activity

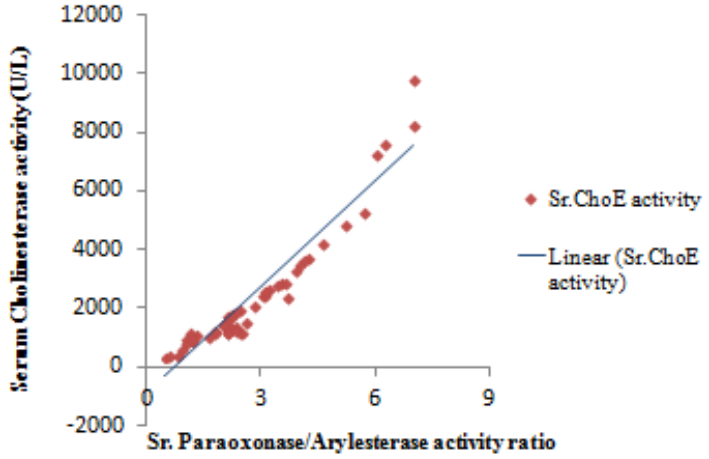

Fig-6: Serum cholinesterase activity vs paraoxonase to arylesterase activity ratio

DISCUSSION

Acute organophosphorus compound (OPC) poisoning is an emergency medical condition since it causes neurotoxicity by inhibiting cholinesterase enzyme (CHE), especially at neuromuscular junction [5,6]. It has been stated that acute OPC poisoning accounts for approximately 10% of intensive care unit admissions in India [3]. Early diagnosis and assessment of the severity of OPC poisoning are crucial in saving lives for which the estimation of cholinesterase is used to confirm the exposure of OPC. Though cholinesterase activity is used to monitor the course of the disease and to assess the response to treatment, it is not beneficial to predict the blood level of OPC and the susceptibility of a patient to the various types of OPC toxicity[10] for which we need to measure the concentration of OPC in blood. But this could not be performed as a routine procedure.

Paraoxonase1 (PON1) is an enzyme that reduces the blood concentration of active forms of OPC by degrading them [11]. It also decreases the level of oxidative stress which is a major cause for the intermediate syndrome of OPC toxicity. The extent of OPC toxicity will be limited in acute OPC poisoning if the patient has high PON1 activity[17-19].Hence estimation of PON1 activity can be used as an indirect method to assess the concentration of OPC in blood.

Serum Paraoxonase1 also exhibits genotypic and phenotypic polymorphism. It has two different types of enzyme activities namely Arylesterase and Paraoxonase since two alleles contribute to its expression. PON1 obeys the Mendelian codominant inheritance and so it exhibits three types of phenotypes [13,17]. They are homozygous AA, heterozygous AB and homozygous BB with increasing Paraoxonase activity from AA to BB phenotypes [15,20]. This variable level of enzyme activity will affect the detoxifying ability of PON1 and so the blood and tissues concentration of OPC [11,13] and also the severity of the symptoms. Hence the degree of PON1 activity or the protective nature of PON1 has to be assessed by identifying its phenotype in patients with acute OPC poisoning [17,19].

The phenotypic determination of PON1 is useful to assess the degree of susceptibility of a person to OPC toxicity [16] and it will also be helpful to modify the management protocol of an acute OPC poisoned patient[19].

Serum PON1 phenotyping or its activity is mainly influenced by its Paraoxonase activity and less affected by Arylesterase activity as reported by Eckerson., *et al.* [13]. In the previous studies, the phenotyping or its activity was assessed by measuring only the activity of Paraoxonase[12,19]or Arylesterase[20,21] individually or by calculating the ratio of Paraoxonase/Arylesterase activity[13,17,18]. The reliability in the determination of PON1 activity or phenotyping will vary because of these various methods of analysis.

Hence we have conducted this study, on the analysis of these various methods used for the determination of PON1 phenotypes or PON1 activity of an individual and observed their reliability by correlating them with the activity of serum Cholinesterase in 'Acute' OPC poisoning.

We analyzed the activities of paraoxonase by using 'paraoxon' and arylesterase by using 'phenylacetate' as substrates in the estimation procedure. The estimated mean values of non-enzymatic hydrolysis of Paraoxon and Phenylacetate were 3.1U/L and 0.26kU/L. The negligible non enzymatic degradation of these substrates substantiates their use for the enzyme assays. The intra and interassay variability of serum Paraoxonase and Arylesterase activities showed the coefficient of variation of 2.66% and 2.11% respectively and 3.26% and 2.75% respectively. This indicated the stability of enzyme activity of PON1in a given serum sample as observed by Eckerson.,*et al*[17]. .

We found that the mean values of Cholinesterase, Paraoxonase and Arylesterase activities of the study population were 2325U/L, 218.39U/L and 85.13kU/L. These activities were measured after standardizing the procedure with quality control sera.

The Pearson coefficient of correlation and the 'p' value for Paraoxonase activity with Cholinesterase activity was noted as +0.767 and < 0.0001 which indicated the significant and positive association of Paraoxonase activity with cholinesterase enzyme activity. The correlation value of +0.955 and the 'p' value of < 0.0001 for the ratio of PON1 allozyme activities with cholinesterase activity showed a strong significant positive association between them. But the association of Arylesterase activity with cholinesterase activity was observed as weak and negative with a correlation value of -0.239 and also was noted as insignificant with 'p' value of> 0.05.

These results were in line with the observations of Abessolo FA., *et al.* where the substrate 'Paraoxon' is the one which differentiates the two activities of PON1 [20]. This has also been explained as the Paraoxonase activity of serum PON1 is highly dominant than Arylesterase activity which influences the differences in the level of enzyme activity and so the xenobiotic activity of PON1 among the phenotypes as stated by Eckerson.,*et al*[13].

These observations proved that the phenotypic polymorphism of serum paraoxonase1 enzyme is mainly affected by its Paraoxonase activity and so the estimation of Paraoxonase activity is vital for the assessment of PON1 activity of an individual. In this

study, we have noted that the determination of PON1 phenotyping has to be done either with measuring the Paraoxonase activity or the ratio of PON1 allozymes activity.

From the present study, we have also found that the ratio of Paraoxonase/Arylesterase activity of PON1 is the more reliable method for serum PON1 phenotyping since it includes both the variables with higher correlation factor than estimating only the Paraoxonase activity. It was also observed that the estimation of only Arylesterase activity is less reliable for phenotyping and also for assessing PON1 activity.

CONCLUSION

From this analytical study, it can be concluded that the Paraoxonase activity of PON1 and the ratio of its allozymes activities have a significant positive linear association with serum Cholinesterase activity. The Arylesterase activity of PON1 has an insignificant negative correlation with cholinesterase activity. Hence phenotyping of serum paraoxonase1 enzyme can be done either by estimating the Paraoxonase activity of PON1 or by calculating the ratio of Paraoxonase/Arylesterase activities.

The more reliable method for the determination of serum paraoxonase1 enzyme phenotyping and the assessment of PON1 activity was found to be the ratio of allozyme activities of PON1 enzyme which can be used in the prediction of cholinesterase activity in a patient with acute OPC poisoning. However, to explore the clinical utility of this reliable method for serum PON1 phenotyping, the study can be done in a wider population.

REFERENCES

1. Paudyal BP. Organophosphorus poisoning. JNMA; journal of the Nepal Medical Association. 2008;47(172):251-8.
2. Eddleston M. Patterns and problems of deliberate self-poisoning in the developing world. Q J Med. 2000; 93: 715–731.
3. Amit Patil, Rahul Peddawad, Vikas Chandra SahayVerma, HemangiGandhi. Profile of Acute Poisoning Cases Treated in a Tertiary CareHospital: a Study in Navi Mumbai. APJMT. 2014; 3 (1):36-40.
4. Maharani B, Vijayakumari N. Profile of poisoning cases in a Tertiary care Hospital, Tamil Nadu, India. Journal of Applied Pharmaceutical Science. 2013 Jan 1;3(1):91.
5. Dandapani M, Zachariah A, Kavitha MR, Jeyaseelan L, Oommen A. Oxidative damage in intermediate syndrome of acute organophosphorous poisoning. Indian journal of medical research. 2003 Jun 1;117:253-9.
6. Nand N, Aggarwal HK, BhartiK. Organophosphate Induced Delayed Neuropathy- a case report. J Assoc Physicians India. 2007; 55:73.
7. Wadia RS, Shinde SN, Vaidya S. Delayed neurotoxicity after an episode of poisoning with dichlorovos. Neurology India: 1985; 33:247-53
8. Xu C1, Zhang XG, Yang X, He YZ. The Diagnostic value of butyrylcholinesterase in acute organophosphorus pesticide poisoning. 2010Apr; 22(4):193-6.
9. Mastrantonio, G, Mack HG, Della Védova, CO. Interpretation of the mechanism of Acetylcholinesterase inhibition ability by organophosphorus compounds through a new conformational descriptor, An experimental and theoretical study. Molecular Model. 2008; 14(9):813-821.
10. Buckley NA, Eddleston M, Szinicz L. Oximes for acute organophosphate pesticide poisoning. Cochrane Database Syst Rev. 2005 Jan 25; (1):CD005085.
11. Sogorb MA, Vilanova E, Carrera V. Future applications of phosphotriesterases in the prophylaxis and treatment of organophosporus insecticide and nerve agent poisonings. Toxicology letters. 2004 Jun 15;151(1):219-33.
12. Costa LG, Li WF, Richter RJ, Shih DM, Lusis A, Furlong CE. The role of paraoxonase (PON1) in the detoxication of organophosphates and its human polymorphism. Chemico-biological interactions. 1999 May 14;119:429-38.
13. Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. Am J Hum Genet.1983;35: 1126–1138.
14. Agrawal S, Tripathi G, Prajnya R, Sinha N, Gilmour A, Bush L, Mastana SS. Paraoxonase 1 GENE polymorphisms contribute to coronary artery disease risk.
15. Hampe MH, Mogarekar M. Paraoxonase1 activity, its Q192R polymorphism and diabetic retinopathy in type 2 diabetes mellitus. Clin Sci. 2014;98(3):355-63.
16. Costa LG, Richter RJ, Li WF, Cole T, Guizzetti M, Furlong CE. Paraoxonase (PON 1) as a biomarker of susceptibility for organophosphate toxicity. Biomarkers. 2003 Jan 1;8(1):1-2.
17. Eckerson HW, Romson JO, Wyte C, La Du BN. The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. American journal of human genetics. 1983 Mar;35(2):214.
18. Draganov DI, Teiber JF, Speelman A, Osawa Y, Sunahara R, La Du BN. Human paraoxonases (PON1, PON2, and PON3) are lactonases with overlapping and distinct substrate specificities. J Lipid Res 2005; 46:1239-1247.
19. Akgur SA, Ozturk P, Sozmen EY, Delen Y, Tanyalcin T, Ege B. Paraoxonase and acetylcholinesterase activities in humans exposed to organophosphorous compounds. Journal of

Toxicology and Environmental Health part A. 1999 Dec 24;58(8):469-74.

20. Abessolo FA, Bruno MJ, N'negue MA, Yangou M, Ngou-MilamaE.Enzymatic and genetic polymorphisms of paraoxonase-1 in the Gabonese population: the relation to lipid parameters in patients with diabetes. JEMDSA. 2012; 17(2):92-99.
21. Granér M, James RW, Kahri J, Nieminen MS, Syvänne M, Taskinen MR. Association of paraoxonase-1 activity and concentration with angiographic severity and extent of coronary artery disease. Journal of the American College of Cardiology. 2006 Jun 20;47(12):2429-35.