

Original article:

Association of serum paraoxonase1 phenotypes with activity of serum cholinesterase in acute organophosphorus compound poisoning

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ABSTRACT

Introduction: Acute poisoning with organophosphorus compounds is a medical emergency since they cause different grades of neuromuscular toxicity. The severity of the disease depends on the type and concentration of OPC in blood. But the degree of susceptibility of patients and severity are being altered by their metabolic capacity of OPCs which can be assessed by determining their phenotypic activity of paraoxonase1, a polymorphic xenobiotic enzyme that metabolizes OPCs.

Aim & Objectives: To identify the serum Paraoxonase1 (PON1) phenotype and to observe its relationship with serum Cholinesterase (CHE) activity in patients with 'Acute' OPC poisoning for assessing the significance of PON1 phenotype determination.

Methods & materials: Serum cholinesterase activity by kinetic method (DGKC) and PON1 phenotypes AA, AB, BB determination by using anti modes 3.0 & 6.9 reported by Mahesh Harishchandra Hampe et al after measuring its Paraoxonase/Arylesterase activity ratio as per Eckerson., *et al* method and Modified Zeller's method was done in 54 patients admitted with acute OPC poisoning. The Statistical analysis was performed using Statistical software SPSS (version 16) package.

Observation & results: Pearson correlation coefficient (r) value of +0.857 and 'p' value of <0.0001 denoted the strong positive correlation between serum Cholinesterase activity and serum Paraoxonase1 phenotypes among the acute OPC poisoning patients.

Discussion & Conclusion: From this study, it was evident that the levels of serum paraoxonase1 activity of PON1 phenotypes are strongly related to cholinesterase activity. Hence it has been shown that serum PON1 phenotyping is useful in the identification of susceptible patients to OPC toxicity.

Keywords: Organophosphorus poisoning, Paraoxonase1 phenotypes, PON1 polymorphism

INTRODUCTION

Poisoning is one of the prevalent causes of poor health and mortality worldwide. Organophosphorus compounds (OPC) used in agriculture as pesticides are increasing in developing countries like India. Intoxication to OP compounds leads to a major health problem globally¹. It has been reported that approximately 10% of admissions in intensive medical care department in India accounts for acute OPC poisoning². Acute OPC poisoning is a medical emergency since organophosphorus compounds inhibit Acetyl Cholinesterase (AChE) and Neurotoxic Target Esterase (NTE)^{3,4} that result in different grades of neuromuscular toxicity^{5,6}. OPCs also increase the oxidative stress and damage the nervous and muscular tissues^{6,7,8}. Serum Cholinesterase activity is a

sensitive diagnostic alternative for neural Acetylcholinesterase activity. Serial estimation of serum Cholinesterase activity is useful in monitoring the disease progress and grading the severity of toxicity⁹. Although serum Cholinesterase activity has a significant role in monitoring the progression of disease and response to therapy, it could not predict the level of OPC in blood and extent of toxicity^{10,11}.

Paraoxonase1 (PON1) is a xenobiotic enzyme that degrades the active OPC and reduces the oxidative stress which is one of a cause of the intermediate syndrome, thereby PON1 has a protective role in OPC poisoning^{12,13}. Since Paraoxonase1 is a polymorphic enzyme, its phenotypes differ in their level of enzyme activity that will affect the level of OPC in blood and tissues¹⁴ and the severity of a disease.

The present study was undertaken to study and evaluate the relationship between the enzyme Cholinesterase that is inhibited by OPCs and the enzyme Paraoxonase1 that detoxifies the OPCs in 'Acute' OPC poisoning.

AIM

To identify the serum Paraoxonase1 (PON1) phenotype and its relationship with serum Cholinesterase activity in patients with acute Organophosphorous compound (OPC) poisoning.

OBJECTIVES

1. To determine the phenotype of serum Paraoxonase1 (PON1) and to estimate the serum Cholinesterase enzyme activity in patients with acute OPC poisoning.
2. To assess the correlation between the phenotypic enzyme activity of serum Paraoxonase1 and serum Cholinesterase activity in acute OPC poisoning.
3. To establish the need for identification of serum Paraoxonase1 phenotype in patients with acute OPC poisoning for the early detection of their susceptibility to OPC toxicity.

MATERIALS AND METHODS

This observational study was conducted in 24 hours clinical biochemistry laboratory, Govt. Stanley Medical College and Hospital, Chennai where the clinical data and samples were collected from 54 patients aged between 18-57 years with acute OPC poisoning, who were admitted within 24 hours of exposure in Intensive Care Unit, Govt. Stanley Hospital, Chennai over a period of 6 months. The study was commenced with the approval obtained from the Institutional Ethics Committee, Govt. Stanley Medical College, Chennai. Informed consent was obtained from all the patients who participated in this study.

The serum Cholinesterase activity was measured by kinetic method as per the recommendation of DGKC using Transasia Biomedicals reagent kit in day 1, 3 and 5 samples and the average value was taken for statistical analysis. The phenotypes of serum PON1 of OPC poisoning patients were determined by calculating the ratio of PON1 allozyme activities such as Paraoxonase and Arylesterase activities¹⁴.

Serum Paraoxonase activity and serum Arylesterase activity of PON1 were measured by Eckerson., *et al* method^{12,14} and Modified Zeller's method^{12,14} respectively using Sigma-Aldrich chemicals. The estimation methods for all the three enzymatic activities mentioned above were standardized with quality control sera (QC) obtained from healthy individuals who attended the Master health checkup. Remaining samples were stored at -20°C to assess inter-assay variability.

Table.1: REFERENCE RANGE FOR PHENOTYPIC CLASSIFICATION OF SERUM PON1¹⁵

Phenotype of serum Paraoxonase1	Ratio of PON 1 allozymes activity	Grades of Serum PON1 Activity
AA	< 3.0	Low
AB	3.0 – 6.9	Moderate
BB	>6.9	High

All the biochemical data of 54 patients with acute OPC poisoning were set for statistical analysis which was performed using Statistical software SPSS (version 16) package. The comparison of mean values of variables among more than two independent groups was analyzed by ‘one way ANOVA’ test. The level and strength of correlation between serum paraoxonase1 phenotypes and serum Cholinesterase activity were calculated using ‘Pearson coefficient of correlation’. A p value of ≤ 0.05 was considered as statistically significant. The results of the statistical analysis were presented as tables and charts.

RESULTS

Serum Paraoxonase and Arylesterase activities were estimated to determine PON1 phenotypes of study population. Serum cholinesterase (CHE) activity was measured to analyze the relationship of CHE with serum PON1 phenotypes. Majority of the study population were in the age group of 18-37 years (Table.2) and 63% were males (Figure.1). The phenotypic polymorphism of serum paraoxonase1 enzyme was shown by the activities of its allozymes namely Paraoxonase and Arylesterase (Figure.2).The trimodal distribution of PON1 phenotypes among OPC poisoning patients were represented by the anti modes at 3.0 and 6.9 as AA, AB, BB respectively (Figure.3). 64.8% of study population was having AA phenotype of serum PON1 enzyme (Figure.4). The mean values of cholinesterase activity amongst PON1 phenotypes AA, AB and BB were 1227.4, 3794.9 and 9052.5 respectively (Table.3). The relationship of the activities of serum cholinesterase was positive and linear with serum PON1 phenotypes of study population (Figure.5). Pearson correlation coefficient value for serum Cholinesterase activity and serum Paraoxonase1 phenotypes was +0.857 with ‘p’ value of <0.0001 (Table.4).

Table.2: AGE DISTRIBUTION AMONGST OPC POISONING PATIENTS

Age in years	No. of patients	Percent (%)
18-27	18	33.4
28-37	20	37
38-47	8	14.8
48-57	8	14.8

Figure.1: GENDER DISTRIBUTION OF OPC POISONING PATIENTS

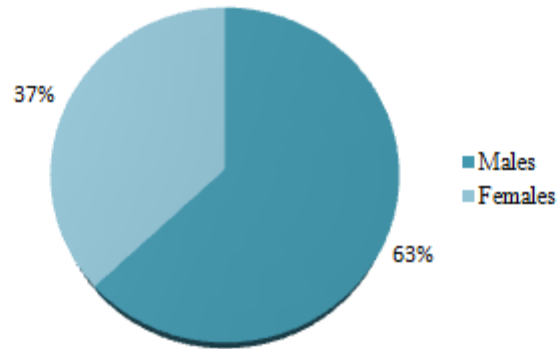


Figure.2: SERUM PARAOXONASE1 PHENOTYPIC POLYMORPHISM

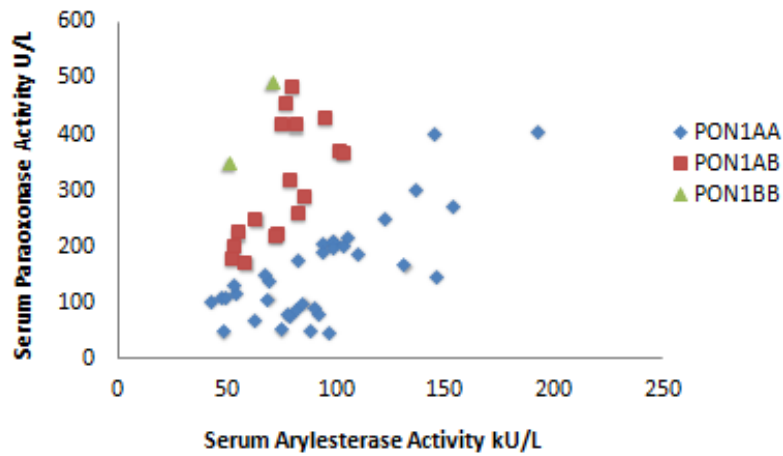


Figure.3: TRIMODAL POPULATION DISTRIBUTION OF SERUM PON1 PHENOTYPES AMONGST OPC POISONING PATIENTS AT 3.0 AND 6.9

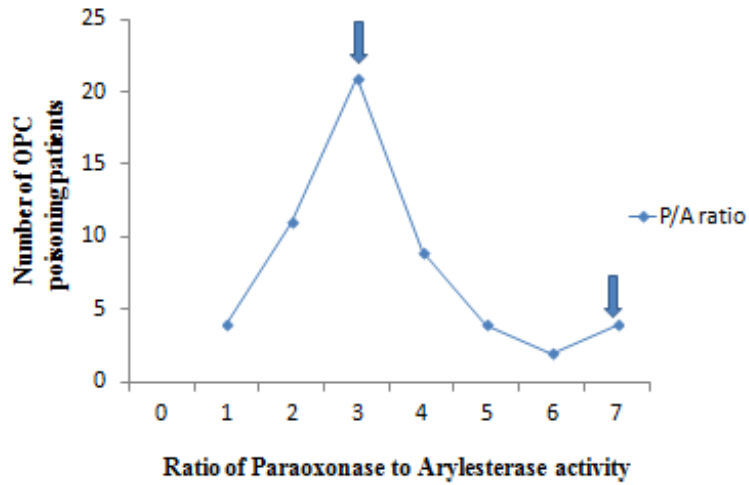


Figure.4: DISTRIBUTION OF PON1 PHENOTYPES AMONGST OPC POISONING PATIENTS

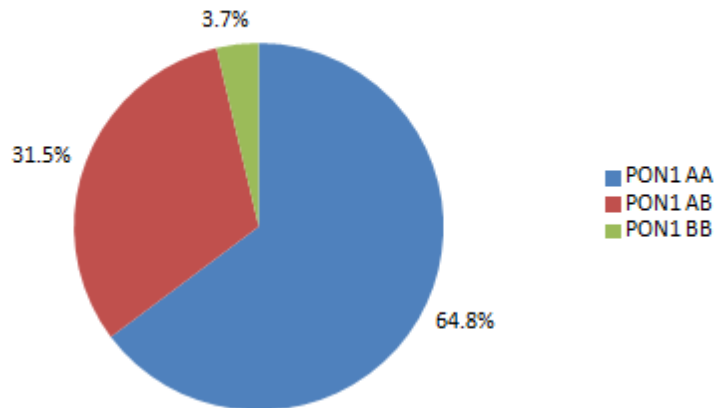


Table.3: MEAN VALUES OF CHOLINESTERASE ENZYME ACTIVITIES AMONG SERUM PON1 PHENOTYPES OF THE STUDY POPULATION

Serum Enzyme activity	PON1AA	PON1AB	PON1BB
Cholinesterase (U/L)	1227.4	3794.94	9052.5

Figure.5: RELATIONSHIP OF SERUM CHOLINESTERASE ACTIVITIES AMONGST PON1 PHENOTYPES OF STUDY POPULATION.

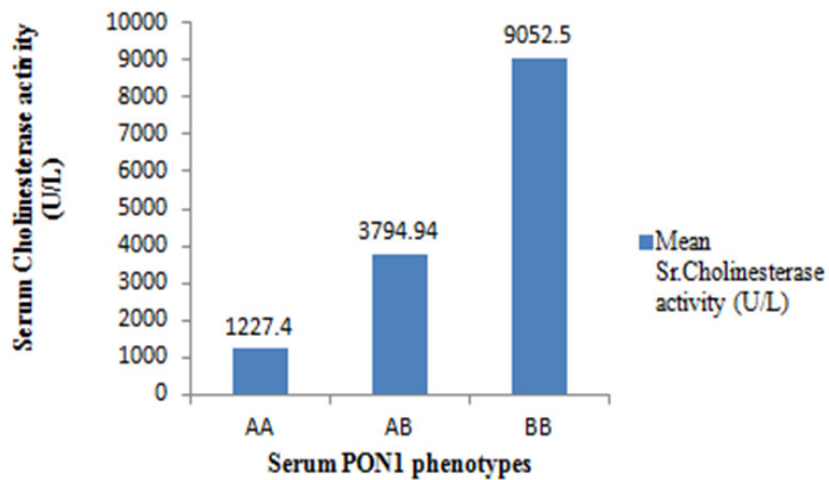


Table.4: CORRELATION OF SERUM CHOLINESTERASE ACTIVITY AND SERUM PARAOXONASE1 PHENOTYPES

Variables	Coefficient of Correlation (r)	Level of Significance (p)	Strength of Association	Interpretation
Sr.Cholinesterase vs Sr.PON1 phenotypes	+0.857	< 0.0001	Strong association	Significant with positive correlation

DISCUSSION

Organophosphorus (OPC) poisoning whether intentional or accidental is one of the leading causes of morbidity and mortality worldwide especially in developing countries like India^{16,17}. Acute OPC poisoning is a clinical emergency in which the early diagnosis and treatment are crucial¹⁸. The main mechanism of OPC toxicity involves the inhibition of Cholinesterase and Neuropathy Target Esterase activity (NTE) and activation of oxidative damage which leads to different grades of neuromuscular toxicity and various stages of paralysis which occur in a different time interval.^{3,4,7,8}.

The exposure to OPCs can be diagnosed early by the detailed history and clinical features of OPC poisoning and confirmed by biochemical analysis of Cholinesterase activity and urine metabolites of OPCs⁸. The toxicity level of OPC poisoning can be detected by the direct measurement of the blood level of OPCs which is practically difficult to follow as a routine procedure. Though RBC cholinesterase is specific to confirm the exposure to OPCs, Serum Cholinesterase activity is a sensitive diagnostic alternative for neuronal AChE activity¹⁹ and has an advantage in monitoring the disease progression during management by serial estimation of enzyme activity⁹. In spite of the advances in diagnosis and treatment of OPC poisoning, the outcome is still guarded because of the three different phases of the poisoning¹⁸. Although the acute cholinergic crisis (Type I paralysis) is the earliest manifestation of acute organophosphorus compound poisoning, it is the intermediate syndrome (Type II paralysis) which has a high degree of mortality⁵. Some patients may develop OPC induced delayed neuropathy (OPIDN), the type III paralysis which usually occurs after 10 days of exposure^{7,8}.

It has been postulated that there is a phenomenon called "re-inhibition of oxime reactivated acetyl cholinesterases" due to a persistently high concentration of OPC which nullifies the effectiveness of oximes¹⁰. Hence the severity of OPC poisoning is decided by the degree and duration of inhibition of cholinesterase activity. It depends on the level of active oxon forms of OPC and is directly related to the activity of serum Paraoxonase I enzyme that detoxifies the active OP compounds. These findings have been found to be reported in chronic OPC poisoning studies^{12,20}. Sogorb MA., *et al* conducted studies in experimental animals and reported that the blood and tissue concentrations of organophosphates were lowered by the action of mammalian Paraoxonase I which has a beneficial role in OPC poisoning^{11,21}. Since the serum PON1 is a polymorphic enzyme, the inherent detoxifying ability is highly variable among individuals and it affects the susceptibility of a patient to OPC toxicity^{20,22}. Eckerson HW, *et al* reported that the trimodal distribution of serum PON1 phenotypes can be identified by the ratio of allozyme activities of PON1¹⁴.

In the present study, we assessed the relationship of serum PON1 phenotypes with serum cholinesterase activity among 'Acute' OPC poisoned patients. We also evaluated the socio demographic profile to assess the pattern of OPC poisoning in the area where the study was conducted. It has been observed that most of the study population belongs to the productive age group of 18 – 37 years and 63% of the patients were males. The individual allozyme activities of serum PON1 enzyme namely paraoxonase and arylesterase were measured using the substrates 'Paraoxon' and 'phenylacetate' in vitro. It has been noticed that the ratio of paraoxonase and arylesterase activities of serum PON1 has proved the presence of the phenotypic polymorphism of PON1 enzyme which is contributed by two alleles¹⁴. The phenotypic polymorphism was also revealed by the trimodal distribution of the serum PON1 enzyme as having three degrees of enzyme activities. The existence of three phenotypes of serum PON1 such as AA, AB and BB were observed at the level of anti modes 3.0 and 6.9 which has been recently reported in India by Mahesh Harishchandra Hampe., *et al* (2014)¹⁵.

From the present study, we have observed that PON1 AA, PON1 AB, PON1 BB phenotypes have low, intermediate and high paraoxonase/arylesterase activity ratio respectively as reported by Eckerson., *et al*^{12,14}. It represented that PON1 phenotypes have increasing in order of Paraoxonase activities or their detoxifying abilities from PON1 AA to PON1BB as stated by Abessolo FA., *et al*^{15,23}. The distribution of PON1 phenotype is highly variable worldwide²⁴. In the present study, PON1 AA phenotype was found to be the predominant form and accounts for 64.8% of study population whereas the PON1 AB and BB phenotypes were found in 31.5% and 3.7% of patients respectively. We observed that the level of cholinesterase activity was low in PON1 AA phenotype as like the ratio of serum paraoxonase/arylesterase activity of PON1 enzyme. PON1 AB has an intermediate activity while PON1 BB has the highest level of paraoxonase/arylesterase ratio and cholinesterase activity as found by Akgür SA., *et al* in chronic OPC poisoning²⁰. From the present study, it has been observed that the Pearson correlation coefficient of Serum cholinesterase activity among the serum PON1 phenotypes was +0.857 with a 'p' value of <0.0001. This indicated the strong positive linear relationship between serum cholinesterase activity with PON1 phenotypic polymorphism.

It was evident that serum PON1 phenotype AA has less ability to detoxify the OPCs due their low level of paraoxonase activity leads to increased inhibition of CHE thereby the individuals with PON1 AA were more susceptible to OPC toxicity¹⁵.

CONCLUSION

From the present study, it can be concluded that Phenotypic polymorphism of Serum Paraoxonase I has a strong association with serum Cholinesterase activity thereby has an influence on the patient's susceptibility to OPC toxicity in Acute organophosphorus compound poisoning. Serum PON1 AA phenotype has low paraoxonase activity with low serum Cholinesterase activity. Serum PON1 BB phenotype has high paraoxonase and Cholinesterase activity.

This relationship supports the need for identification of serum Paraoxonase I phenotype along with the measurement of serum Cholinesterase activity in patients with acute OPC poisoning which will help the early detection of their susceptibility to OPC toxicity. Hence the dosage and duration of oxime therapy and the time period for follow up management can be modified according to their susceptibility so as to ensure better prognosis. However to explore the clinical utility of serum PON1 phenotyping in acute OPC poisoning patients, the association of cholinesterase activity and PON1 phenotype polymorphism has to be studied further with the level of OPC in blood and/or urine in a wider population.

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