Original Study

Study of Acetyl Cholinesterase, Butyryl Cholinestrase and β - Glucuronidase in Organophosphorus Poisoning

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Abstract

Organophosphorus poisoning is common problem throughout the world. It occurs due to accidental exposure; suicidal and homicidal attempt. Many deaths occur within hours of ingestion of organophosphorus compounds like pesticides. For its prevention, speedy diagnosis and prompt treatment is required; which requires sensitive marker/s. The aim of this study was to find such marker/s. In this regard, activities of Acetylcholinesterase, Butyrylcholinesterase and β-glucuronidase were estimated in 80 samples including 40 controls and 40 organophosphorus poisoning cases (mild = 07, moderate = 19 and severe = 14). Results indicated that activities of Acetylcholinesterase and Butyrylcholinestrase decrease in mild, moderate and severe Organophosphorus poisoning in proportionate manner whereas, β-glucuronidase activity increases as severity of Organophosphorus poisoning progresses. Thus, all the three enzymes showed alterations in their activities however, the degree of change in activity was maximum in case of Acetylcholinesterase. Thus, Acetylcholinesterase activity is the most sensitive marker amongst three enzymes in Organophosphorus poisoning.

Introduction

Organophosphorus (OP) compounds are widely used in various insecticides/ pesticides, for a variety of purpose in agriculture, human and veterinary medicine. Acute poisoning with OP in human remains one of the major health issue in developed and especially developing countries like India and its frequency increased over years[14]. Poisoning occurs as a result of use of agricultural pesticide, accidental exposure, suicidal and/or homicidal attempts[2,3,4,5].

Organophosphorus compounds have many toxicological effects on the body, some are respiratory disorders, hepatological disorders, cardiovascular disorders, neurological disorders, hormonal imbalance, esophageal effects, renal impairment, altered antioxidant status and increased oxidative stress[6]. These compounds are potent inhibitors of many enzymes having serine as a conserved active site, include Acetyl and Butyryl cholinesterase, Acylpeptide hydrolase, Carboxylesterase, Trypsin, Chymotrypsin, Thrombin, etc[1].

Inhibition of Acetyl Cholinesterase and Butyryl Cholinesterase have an established diagnostic value in Organophosphorus poisoning; along with these β-glucuronidase is to be validated as a marker in the diagnosis and prognosis of Organophosphorus poisoning.

To understand the mechanism and relation of change in the activities of Acetyl Cholinesterase, Butyryl Cholinesterase and β – glucuronidase with Organophosphorus poisoning and to check their sensitivity in Organophosphorus

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poisoning, this study was carried out.

Material and Methods

The study was conducted in the Department of Biochemistry, Dr. V. M. Govt. Medical College, Solapur, in collaboration with Department of Medicine, SCSM General Hospital, Solapur (Maharashtra).

Total 80 samples were analyzed comprising of 40 patients suffering from Organophosphorus poisoning and 40 age and sex matched healthy controls. The Organophosphorus poisoning was diagnosed by the physician on the basis of symptoms, history and according to criteria of World Health Organization (WHO), distribution of Organophosphorus poisoned patients were done into three grades as mild, moderate and severely poisoned patients on the basis of symptoms and % inhibition of Acetyl cholinesterase activity[7].

Distribution of subjects

Group	No. of Subjects
1) Healthy Controls	40
2) Patients with Organophosphorus poisoning	40

Statistical analysis:

The data is expressed as Mean ± SD. The statistical significance of the results was analyzed using a non-parametric Tukey's test (for unequal sample size).Values of $p<0.001$ were considered significant. Correlations between the parameters were also evaluated in all study subjects and control group and were calculated by Pearson's method.

Exclusion criteria:

Patients with Organochlorine poisoning, Organometallic poisoning, Organonitrile poisoning, food poisoning, snake poisoning were excluded from the study group.

Collection of blood samples:

The blood samples for the study were collected in a heparin bulb before giving any kind of therapy especially an injection of atropine.The obtained blood samples were centrifuged to obtain plasma and cells and analysis were done immediately by using separated plasma and RBCs lysate.

Acetyl Cholinesterase (AChE) and Butyryl Cholinesterase (BuChE) estimated by Kinetic colorimetric method, the kit obtained from Siemens healthcare diagnostics Ltd., India[8]. Estimation of β-Glucuronidase (BG) was done by using Gilbert -Goldstein method[9].

Results and Discussion

Organophosphorus poisoning is two edges weapon; ignorance about its toxic effects leads to careless handling and accidental ingestion leading to its poisoning. On the other hand knowledge of its toxic effect leads to its purposeful intake for suicidal attempt progressing to its poisoning.

Enzymatic status in mildly OP poisoned patients (Group I) :

The activities of Acetyl Cholinesterase and Butyryl Cholinesterase were significantly decreased ($p < 0.01$) whereas β-glucuronidase activity was increased significantly ($p < 0.01$) in mildly OP poisoned patients

Table 1
Distribution of patients according to the severity of organophosphorus poisoning

Sr. No.	Grade of poisoning	Symptoms	Inhibition of Acetyl cholinesterase activity in terms of %	No. of Cases
1.	Mild	Nausea, Vomiting, diarrhea, Sweating etc.	Up to 60 %	07
2.	Moderate	Lacrimation, salivation uriosis, fasciculation etc.	60 - 80 %	19
3.	Severe	Apnoic spells, areflexia, ARDS, seizures, coma etc.	> 80 %	14

Table 2

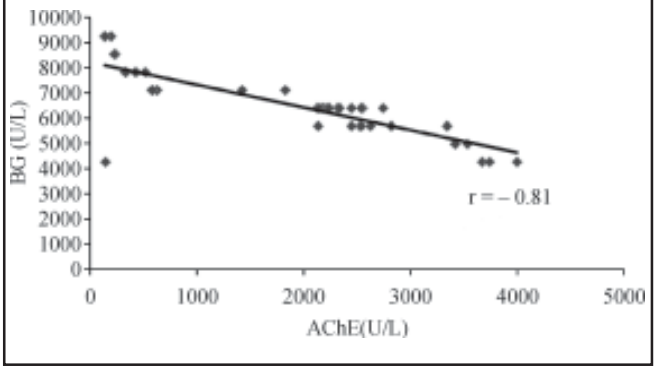
Changes in the activity of Acetyl Cholinesterase, Butyryl Cholinesterase, and β-glucuronidase in blood of Organophosphorus poisoning patients according to severity of poisoning in comparison with control subjects

	Control (n=07) Mean ± SD	Mild OPP Mean ± SD	↓%	↑%	Moderate OPP Mean ± SD	↓%	↑%	Severe OPP Mean ± SD	↓%	↑%
RBC AChE (U/L)	10083.94 ± 721.93	3576.46 ± 240.07	64.53	–	2321.73 ± 327.77	76.97	–	345.36 ± 176.57	96.57	–
Plasma BuChE (U/L)	9138.02 ± 227.34	3756.77 ± 240.45	58.88	–	2528.43 ± 276.55	72.33	–	532.21 ± 230.24	94.17	–
Plasma BG (U/L)	3124.96 ± 385.76	4880.91 ± 385.76	–	35.97	6278.12 ± 450.44	–	50.22	7856.25 ± 1283.75	–	62.22

Data expressed as mean ± SD; AchE - AcetyI cholinesterase; BuCh - Butyryl cholinesterase; BG - β - glucuronidase; ↓% - Decreased Percentage as compared to control mean; ↑% - Increased Percentage as compared to control mean

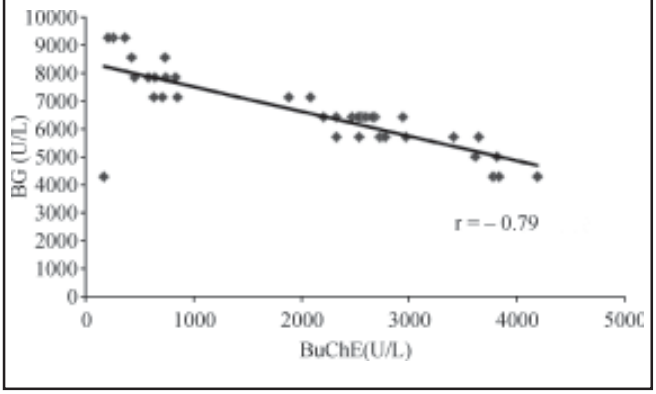
Scatter Diagram 1

Correlation between Acetyl Cholinesterase (AChE) and β-glucuronidase (BG) actively in all organophosphorus poisoning patients



Scatter Diagram 2

Correlation between Butyryl Cholinesterase (BuChE) and β-glucuronidase (BG) actively in all organophosphorus poisoning patients



(Group I) as compared to healthy controls. The Acetyl Cholinesterase activity was almost decreased by 64.53% (2.82 fold) while Butyryl Cholinesterase activity was lowered by 58.88% (2.43 fold) and β-glucuronidase activity was 35.97 % (1.56 fold) (**Table 2**) higher in mild poisoned patients (Group I) as compared to those in healthy controls.

Thus, it was found that Acetyl Cholinesterase is more sensitive to Organophosphorus compounds in case of mild OP poisoning as compared to Butyryl Cholinesterase and β-glucuronidase.

Enzymatic status in moderately OP poisoned patients (Group II) :

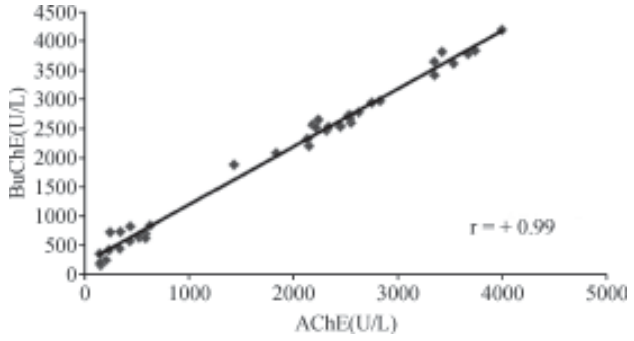
As compared to healthy controls and mildly poisoned patients (Group I), the activities of Acetyl Cholinesterase and Butyryl Cholinesterase were significantly lowered ($p < 0.01$) and β-glucuronidase activity was significantly higher ($p < 0.01$) in moderately poisoned patients (Group II).

Activities of Acetyl Cholinesterase and Butyryl Cholinesterase were decreased by 76.97% (4.34 fold) and 72.33% (3.61 fold) respectively and about 50.22% (2.0 fold) (**Table 2**) increase was seen in β-glucuronidase activity in moderately poisoned patients (Group II) as compared to healthy controls.

Thus, it was observed that both Acetyl Cholinesterase and Butyryl Cholinesterase are sensitive markers in

Scatter Diagram 3

Correlation between Acetyl Cholinesterase (AChE) and Butyryl Cholinesterase (BuChE) actively in all organophosphorus poisoning patients



moderately poisoned patients whereas, β-glucuronidase is less sensitive in this phase.

Enzymatic status in severely OP poisoned patients (Group III) :

As compared to healthy controls and moderately poisoned patients (Group II), the activities of Acetyl Cholinesterase and Butyryl Cholinesterase were significantly lowered ($p < 0.01$) and β-glucuronidase activity was increased significantly ($p < 0.01$) in severely poisoned patients (Group III).

Highly significant lower activities of Acetyl Cholinesterase and Butyryl Cholinesterase 96.57% (29.2 fold) and 94.17% (17.16 fold) respectively and 60.22% (2.51 fold) (**Table 1**) higher activity of β-glucuronidase were seen in severely poisoned patients (Group III) when compared with healthy controls.

Thus, it was found that Acetyl Cholinesterase activity is more sensitive in severe poisoning as compared to activity of Butyryl Cholinesterase and β-glucuronidase.

The activities of Acetyl Cholinesterase and Butyryl Cholinesterase are inhibited by the Organophosphorus compounds. Organophosphorus compound binds to hydroxyl group of serine residue present at active site of enzyme, this reaction is called as phosphorylation of enzyme and this phosphorylated enzyme is catalytically inactive. This reaction proceeds via an intermediate Michaelis–type complex between the enzyme and Organophosphorus compound. Phosphorylation of serine esterases by OP compounds occurs on a 1:1 molar basis. Thus, when serine residue of enzyme present at enzyme's catalytic site is phosphorylated, it is not available to catalyze the reaction, thus enzyme gets inhibited[10,11].

It is seen that, there is weak interaction between Acetyl choline and Butyryl Cholinesterase. Thus, Acetyl Cholinesterase is predominant enzyme which hydrolyzes acetylcholine at synapses[12]. Organophosphorus compounds covalently block the active site of serine residue of Acetyl Cholinesterase by undergoing nucleophilic attack to produce a serine-phosphoester adduct. This irreversible inactivation lead to an overstimulation of acetylcholine receptors and thereby excess accumulation of acetylcholine in peripheral and CNS causing cholinergic manifestations such as vomiting, meiosis, hyper-salivation, respiratory distress, abdominal pain, depressed level of consciousness and muscle fasciculation[13].

In the present study, we have also studied changes in the activity of β-glucuronidase in different groups of Organophosphorus poisoning patients.

The enzyme β-glucuronidase is present in hepatocytes in association with an isozyme of carboxylesterase known as Egasyn. Egasyn is serine esterase and in Organophosphorus poisoning, organophosphorus compound binds with egasyn and the complex of microsomal β-glucuronidase and egasyn is dissociated. Egasyn bound to OP compound remains within the hepatocytes and β-glucuronidase is secreted in plasma leading to increase in its activity. Thus, increased plasma activity of β-glucuronidase can be a measure of Organophosphorus poisoning[15,16,17,18].

Concomitant decrease in the activities of the enzymes, namely Acetyl Cholinesterase and Butyryl Cholinesterase represented significant positive correlation ($r = +0.99$) and a significant negative correlation between Acetyl Cholinesterase and β-glucuronidase was seen in ($r=-0.81$) Organophosphorus poisoning patients (not in severe poisoned i.e. group III patients) depicting substantial change in the activities of these enzymes with the increased Organophosphorus exposure/ poisoning. Similar correlation was perceived between Butyryl Cholinesterase and β-glucuronidase ($r=-0.79$) showing proportionate change in the activities of these enzymes in OP patients.

Thus, from correlation study and alterations observed in the activities of studied enzymes in Organophosphorus poisoning, alleviations in the activities of Acetyl Cholinesterase, Butyryl Cholinesterase and increase in β-glucuronidase activity is linear as organophosphorus poisoning progresses. If the amount of change in activities is compared it can be seen that in mild and moderate Organophosphorus poisoning, all the three enzymes showed change in activities to more or less extent. However, in all stages of Organophosphorus poisoning, the decrease in the activity of Acetyl Cholinesterase is maximum as compared to the activities of Butyryl Cholinesterase and β-glucuronidase.

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Organophosphorus poisoning is two edges weapon; ignorance about its toxic effects leads to careless handling and accidental ingestion leading to its poisoning. On the other hand knowledge of its toxic effect leads to its purposeful intake for suicidal attempt progressing to its poisoning.

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Thus, it was found that Acetyl Cholinesterase is more sensitive to Organophosphorus compounds in case of mild OP poisoning as compared to Butyryl Cholinesterase and â-glucuronidase.

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Thus, it was observed that both Acetyl Cholinesterase and Butyryl Cholinesterase are sensitive markers in moderately poisoned patients whereas, â-glucuronidase is less sensitive in this phase.

Enzymatic status in severely OP poisoned patients (Group III) :-

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