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Xanthine Oxidase and Paraoxonase – 1 as a new markers in the diagnosis and prognosis of organophosphorus pesticide poisoning

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Abstract :

Background: Acetylcholinesterase is already well known marker for organophosphorus poisoning in human beings. But, our interest was to known for another new markers. There is high limited study on xanthine oxidase and paraoxonase 1 in human beings having organophosphorus poisoning. Thus, aim of our this research was to estimate and correlate activities of xanthine oxidase and paraoxonase 1 in different stages of organophosphorus poisoning in a way to find out potential of these enzymes as marker of organophosphorus poisoning in human beings.
Method: Xanthine oxidase (XO) activity was determined by Roussos method while paraoxonase (PON1) activity was determined by spectrophotometric method.
Result: As compared to healthy controls, the activities of paraoxonase 1 were continuously and significantly decreased **($p < 0.01$)** from group I to group V, while, the activities of xanthine oxidase were continuously and significantly increased **($p < 0.01$)** from group I to group V in organophosphorus poisoned patients.
Conclusion: We found a proportional increase in xanthine oxidase (XO) and a proportional decrease in paraoxonase 1 activity with severity of organophosphorus poisoning. Increase in xanthine oxidase activity is less significant compared to decrease in activity of paraoxonase 1. However, xanthine oxidase activity is a marker of oxidative stress developed in organophosphorus poisoning. Thus, paraoxonase 1 (PON1) can be used as a potent biochemical marker in the diagnosis and prognosis of organophosphorus poisoning along with xanthine oxidase (XO).

Keywords: Paraoxonase, xanthine oxidase, cholinesterase, xanthine dehydrogenase, organophosphorus.

1 Introduction

Pests are the animals or plants which are harmful to the interest of human beings. The term pest include insects, rodents, nematodes, fungi, bacteria, weeds and various parasitic plants. Viruses and disease causing microorganisms not belongs to this category.[1] A pesticide is a substance or mixture of substances used to kill a pest. The term pesticide is defined as, any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs. [2]
The term pesticides include insecticides, herbicides, fungicides, rodenticides and disinfectants. A pesticide may be a liquid (mist or spray), solid (dust, granule or bait) or gas (vapour) applied to control unwanted insects, ticks, mites, plants or animals in a given area.[3]
Further, pesticides are broadly classified into organophosphorus pesticides, organochlorine pesticides, carbamates, pyrethroids etc.[4] Organophosphates are traditionally used because of their effectiveness against a variety of pests and because the pests do not appear to develop resistance to this class of pesticides as they do to others. Organophosphorus pesticides are very powerful neurotoxins resulting in neurotoxicity on acute or chronic exposure.[5]
Organophosphorus pesticide poisonings remain a serious public health problem worldwide. According to the World Health Organization's estimate, 30,00,000 cases of pesticide poisoning occur every year, resulting in more than 2,50,000 deaths.[6,7]
Organophosphorus compounds inhibit the function of carboxylic ester hydrolases such as chymotrypsin, acetyl cholinesterase, plasma or pseudocholinesterase (butyryl cholinesterase), plasma and hepatic carboxylesterases (aliesterases), paraoxonases (asterases) and other nonspecific esterases within the body. Organophosphates can bind into the acyl pocket at the active site of acetyl cholinesterase. The binding of a phosphate (organophosphate) group to

the serine amino acid at the active site of acetyl cholinesterase changes the configuration of the enzyme molecule, stabilizing it and preventing it from functioning.[8]
Inhibition of acetyl cholinesterase activity leads to the accumulation of acetylcholine at the synapses causing overstimulation of both central and peripheral nervous systems.[9,10] Thus, exposure to organophosphorus compounds interferes synaptic transmission peripherally at muscarinic and nicotinic receptors.[11] Overstimulation of these receptors are associated with severe symptoms of poisoning. Level of acetyl cholinesterase inhibition and severity of poisoning symptoms in humans increases with increased pesticide doses, exposure time, atmospheric temperature, developmental age and sex.[12]
Thus, a progressive decrease in acetyl cholinesterase activity is proportional to increase in severity of poisoning and it reflects proportionate amount of pesticide consumed and absorbed.[13]
Paraoxonases are a group of enzymes involved in the hydrolysis of aromatic carboxylic acid esters and organophosphorus insecticides. Paraoxonases (aryl-dialkyl-phosphatase (EC 3.1.8.1) are serum esterases that are synthesized by the liver. Many studies have indicated the existence of a genetic polymorphism of paraoxonase. This polymorphism is due to an amino acid substitution in the active site of the enzyme, giving rise to low and high activity isoenzymes. The resulting polymorphic variation in serum paraoxonase activity may affect the metabolism of organophosphates in individuals at risk of exposure and therefore increase the risk of acute organophosphate intoxication or of organophosphorus-induced delayed polyneuropathy. [14,15,16,17]
Xanthine oxidase is a form of xanthine oxidoreductase, an enzyme that generates reactive oxygen species. It catalyzes the conversion of hypoxanthine to xanthine and of xanthine to uric acid. This enzyme plays an important role in the catabolism of purines in some species, including humans. [18,19,20,21]
Acetylcholinesterase is already well known marker for organophosphorus poisoning in human beings. But, our interest was to known for another new markers. There is high limited study on xanthine oxidase and paraoxonase 1 in human beings having organophosphorus poisoning. Thus, aim of our this research was to estimate and correlate activities of xanthine oxidase and paraoxonase 1 in different stages of organophosphorus poisoning in a way to find out potential of these enzymes as marker of organophosphorus poisoning in human beings.

2 Materials and methods

Subjects: The study was conducted at Shri. Chhatrapati Shivaji Maharaj General Hospital, Solapur and Dr. V M Medical College, Solapur during the period January - 2008 to Dec- 2009. We have studied total 60 organophosphorus poisoned patients and 40 controls. Organophosphorus poisoned patients were identified by attending physicians on the basis of symptoms shown by patients. Symptoms such as hyper-salivation, convulsions, respiratory failure, ataxia, slurred speech, miosis, muscle cramping suggest about organophosphorus poisoning. However, to access organophosphorus poisoning, it is necessary to analyze biological samples mostly blood and/or urine. Organophosphorus compounds can be detected in urine however, their degradation is rapid and hence their detection in urine is possible only within short time. The detection of metabolites of organophosphorus compounds is another way to detect organophosphorus poisoning. Metabolites circulate for longer time and mostly excreted in urine. On other hand, detection of metabolites being useful is time consuming and non-specific procedure because severity of poisoning cannot be assessed from such metabolites.[22] Thus, most commonly, detection of organophosphorus poisoning is done by estimating activities of enzymes namely acetylcholinesterase, butyrylcholinesterase etc. from blood.
Collection of samples: Blood samples of organophosphorus poisoned patients were collected from ICU of Shri. Chhatrapati Shivaji Maharaj General Hospital, Solapur at the time of admission and before treatment with atropine plus PAM. 40 samples of controls were collected from Damani Blood Bank. Patients with any other type of poisoning than organophosphorus poisoning were excluded from the study group. Individuals with any disease/s and similarly, sprayers and individuals working in the vicinity of different pesticides and chemical industries were excluded from the study.
Total 5 ml of venous blood were collected in a heparin bulb. The obtained bloods were centrifuged at 3000 rpm for 10 minutes to obtain plasma and cells. The cells were washed with normal saline for 2 to 3 times and then subjected to lysis by adding ice cold distilled water. The analyses were done immediately by using separated plasma and RBCs lysate.
Estimation of xanthine oxidase : Assay of xanthine oxidase was carried out according to the Roussos method. In an assay tube 300 µl Tris-HCl(50 mM, pH 7.4), 300 µl copper sulphate (10 mM), 50 µl xanthine (15 mM) was taken. The reaction was initiated by adding 100 µl of serum. The absorbance was read at 290 nm on spectrophotometer continuously with a time gap of 1 minute. The enzyme activity was measured by µmoles of uric acid formed due to enzymatic reaction in one minute. One unit (U) of enzyme activity is a enzyme activity that liberate one µmole of uric acid in one minute.[23]
Estimation of paraoxonase 1 : Assay of PON1 is carried out as follow. In an assay tube, 750 µl assay buffer (containing 0.125M Tris-HCl and 1.25 M $CaCl_2$), 25 µl serum and 225 µl paraoxon (6 mM in acetone) is taken. The

absorbance was read at 405 nm on spectrophotometer continuously with a time gap of 1 minute. The enzyme activity was measured by µmoles of p-nitrophenol formed due to enzymatic reaction in one minute. One unit (U) of enzyme activity is a enzyme activity that liberate one µmole of p-nitrophenol in one minute.[24]

3 Result and discussion

A present study includes 60 organophosphorus poisoned patients. The distribution of organophosphorus poisoned patients into five grades as very mild poisoned patients, mild poisoned patients, moderate poisoned patients, severe poisoned patients and highly severe poisoned patients were done according to percent inhibition of acetyl cholinesterase activity and criteria of World Health Organization (WHO).**(Table 1)**

In our study, 41 cases of poisoning were found as a result of suicidal attempts while 19 due to accidental events. It is found that organophosphorus pesticide, dimethoate is more commonly used to attempt suicide as it is easily available. Majority of poisoning cases were due to dimethoate. The other cases of poisoning were due to sumethrine, parathion, deltanuthrin, malathion, cypermethrin, chlorpyriphos, tick – 20 etc. Out of 60 patients studied by us, 43 gets cured while 17 patients got expired. The death generally occurred due to respiratory failure. The results were analyzed by using Tukey test for multiple comparison of k population means (unequal sample sizes). 'p' value < 0.01 were considered significant. The results are summarized as follows. **(Table 2 and 3)**

Table 1: Distribution of patients according to severity of poisoning on the basis of WHOs criteria and symptoms shown by patients

Group	Grade of poisoning	Symptoms	Inhibition of acetyl cholinesterase activity in terms of percentage	No. of cases
I	Very Mild	Nausea, vomiting, diarrhea, sweating etc	Less than 20 %	11
II	Mild		More than 20 % but less than 40 %	13
III	Moderate	Lacrimation, salivation, miosis, fasciculation etc.	More than 40 % but less than 60 %	15
IV	Severe	Incontinence, apnoeic spells, ARDS, areflexia seizures, coma etc.	More than 60 % but less than 80 %	12
V	Highly Severe		More than 80 %	09

Table 2 : Activities of paraoxonase 1 (PON 1) in controls and organophosphorus poisoned patients

Parameter	Controls	Group I	Group II	Group III	Group IV	Group V
No. of Cases	40	11	13	15	12	09
Paraoxonase 1 activity (U/ml) Mean ± SD	214.71 ± 5.46	197.26* ± 5.80	171.03* ± 12.41	138.14* ± 18.20	103.95* ± 10.02	59.02* ± 2.59

* P < 0.01 P values < 0.01 are considered significant.

Table 3 : Activities of xanthine oxidase (XO) in controls and organophosphorus poisoned patients

Parameter	Controls	Group I	Group II	Group III	Group IV	Group V
No. of Cases	40	11	13	15	12	09
Xanthine oxidase activity (U/L) Mean ± SD	5.01 ± 0.42	6.90* ± 0.92	9.60* ± 0.65	12.21* ± 1.08	15.96* ± 1.04	20.68* ± 1.30

* P < 0.01 P values < 0.01 are considered significant.

Paraoxonase 1 in organophosphorus poisoned patients : As compared to healthy controls, the activities of paraoxonase 1 were continuously and significantly decreased **(p < 0.01)** from group I to group V in organophosphorus poisoned patients. As compared to healthy controls there was a decrease in paraoxonase activity as, 1.09 times for group I, 1.26 times for group II, 1.54 times for group III, 2.06 times for group IV and 3.64 times for group V. **(Table 2)**

Xanthine oxidase in organophosphorus poisoned patients : As compared to healthy controls, the activities of xanthine oxidase were continuously and significantly increased **(p < 0.01)** from group I to group V in organophosphorus poisoned patients. As compared to healthy controls there was an increase in paraoxonase activity as, 1.38 times for group I, 1.92 times for group II, 2.44 times for group III, 3.19 times for group IV and 4.14 times for group V. **(Table 3)**

Thus, we found a proportional increase in xanthine oxidase (XO) and a proportional decrease in paraoxonase 1 activity with severity of organophosphorus poisoning which will helpful in the diagnosis and prognosis of organophosphorus poisoning.

Paraoxonases are a group of enzymes involved in the hydrolysis of aromatic carboxylic acid esters and organophosphorus insecticides. Paraoxonases (aryl-dialkyl-phosphatase (EC 3.1.8.1) are serum esterases that are synthesized by the liver. There are three known genotypic forms of paraoxonases. They are coded by the PON set of genes as PON1, PON2 and PON3, located on the long arm of chromosome-7. The differences between them lie in their location and activity.

PON1 is synthesized in the liver and transported along with high density lipoproteins (HDL) in the plasma. It functions as an antioxidant.

PON2 is a ubiquitously expressed intracellular protein that can protect cells against oxidative damage.

PON3 is similar to PON1 in activity but differs from it in substrate specificity. Additionally, it is not regulated by inflammation and levels of oxidized lipids.[25,26,27,28]

PON1 is a calcium ion dependent esterase which hydrolyses variety of organophosphorus compounds. In the blood PON1 is closely attached to high-density lipoprotein particles by apolipoprotein A_1. PON1 inhibits oxidation of low density lipoproteins and also hydrolysis lipid peroxidation products. This enzyme is also involved in decreasing superoxide ion formation. Thus, it is an important antioxidant enzyme. Its serum concentration is influenced by inflammatory changes and the levels of serum oxidized low density lipoproteins.

Xanthine oxidase (EC 1.17.3.2) is composed of two identical and independent subunits. They contains three redox centres, where the molybdenum centre appears as a link between xanthine (substrate) and enzyme. The flavin adenine dinucleotide (FAD), a prosthetic group is declared as the site of oxygen free radical formation. This enzyme is distributed in human heart, liver and kidney. The enzyme is also present in intestinal mucosal epithelium and vascular endothelial cells. Under normal physiological condition, xanthine oxidase exists as a precursor enzyme called as xanthine dehydrogenase (EC 1.17.1.4). Under stress, xanthine dehydrogenase activity gets converted to xanthine oxidase by reversible oxidation or irreversible proteolytic modification of sulfhydril groups in the enzyme. Now, Xanthine oxidase catalyzes conversion of hypoxanthine to xanthine and xanthine to uric acid. During this reaction, reducing equivalents are released. These, reducing equivalents are transferred to O_2 and thus H_2O_2 is generated.[29,30,31]

Organophosphorus compounds induce hypoxia in the body. This happens due to respiratory failure during acute cholinergic crisis. The reasons behind respiratory failure are central respiratory depression, respiratory muscle weakness, bronchospasm, bronchorrhoea and peripheral respiratory failure. Under hypoxic condition mitochondrial aerobic metabolism slows down and thus adenosine triphosphate (ATP) formation decreases. Also, failure of calcium pump of cell membrane and organelle membrane takes place. Thus, overload of calcium takes place in cytoplasm of the cell which is responsible for activation of calcium dependent proteolytic enzymes. These proteolytic enzymes now convert xanthine dehydrogenase into xanthine oxidase. This, increase in xanthine oxidase activity led to excessive production of free radicals and hence decrease in antioxidant capacity.[32,33,34]

4 Conclusion

In this study, we found that the serum activity of paraoxonase 1 (PON1) decreases proportionally and significantly and correlates with severity of organophosphorus poisoning. Further, there was found a proportional and significant increase in xanthine oxidase (XO) activity also. Increase in xanthine oxidase activity is less significant compared to decrease in activity of paraoxonase 1. However, xanthine oxidase activity is a marker of oxidative stress developed in organophosphorus poisoning. Thus, paraoxonase 1 (PON1) can be used as a potent biochemical marker in the diagnosis and prognosis of organophosphorus poisoning along with xanthine oxidase (XO).

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