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Correlation of Oxidative Stress to Severity of Acute Organophosphorus Poisoning

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ABSTRACT

Organophosphorus compounds may induce oxidative stress leading to generation of free radicals and alterations in antioxidant and scavengers of oxygen free radicals. The present study included one hundred and sixteen patients who had organophosphorus (OP) poisoning of 3-5 hours duration from the time of consuming the poison. The study focused to elucidate the role of free radicals in organophosphorus toxicity by estimation of various oxidative stress markers. The results of the study suggest significant alteration of these oxidative stress markers which in turn suggests significant free radical generation due to OP poisoning. This may be due to overuse failure of the antioxidant defence system secondary to excessive reactive oxygen species production.
The study also indicated that the incidence of poisoning was more common among males. Overall, the study found that accidental poisoning was far less than suicidal cases with homicidal cases being the rarest. Most of the patients belonged to rural areas.

Key Words: organophosphorus, oxidative stress.

INTRODUCTION

The history of organophosphorus compounds (OP) and their poisonous effect stretches throughout more than a century. Organophosphates and carbamates are used worldwide in pest control. Pesticides are essential for the farmers to ensure a good crop yield. As a result they are available with ease and at a cheap rate everywhere. Most places don't have any restrictions in their supply. As a result they have also become the most commonly used suicidal poisons. They are extremely toxic substances with a very high mortality and morbidity. WHO estimates that about 3 million people are exposed to pesticide poisoning every year and causes about 3,00,000 deaths per year. India has the highest incidence of patients with organ phosphorus poisoning in the world [(1)]. Information regarding poisoning cases in our region is rather limited. Despite an increased incidence of organophosphorus insecticide poisoning, the exact micro molecular changes that take place remain elusive. Our study was directed towards understanding the toxic effects of organophosphorus poisoning on free radical generation and the antioxidant system of the body. In addition demographic factors of poisoning were also analysed.

MATERIAL AND METHOD

The study was conducted at Sher-e-Kashmir institute of Medical sciences Bemina Srinagar which is a tertiary care institute between Sept 2019 to March 2020. Informed consent was obtained from the patients or in some cases was the patient was unable to give consent due to their medical condition, consent was taken from the next of kin. One hundred and sixteen OP poisoned patients (of 3-5 h duration from the time of consuming the poison) were admitted during the said period. The cases included in this study were those who had

consumed the poison orally or had been exposed to it through accidental inhalation during spraying.

The mean age of the patients was 30 ± 5 years. The grouping of the OP poisoning cases was done depending upon signs and symptoms.

Grade I - OP poisoned with no signs and symptoms

Grade II - Diarrhea, vomiting, abdominal pain, giddiness

Grade III - Pupillary constriction with above symptoms

Grade IV - Pulmonary edema

Grade V - Unconsciousness

Immediately after admission to the hospital, before starting the appropriate treatment. 10 ml venous blood samples were collected from the subjects under aseptic conditions.

Assay systems

Protein determination

Protein was determined by the Comassie blue method using bovine serum albumin as standard. Absorbance of samples was measured at 595 nm by method Bradford, 1976. [(2)]

1.2 Acetylcholinesterase activity

AChE activity was assayed by the method of Ellman *etal.* 1961 [(3)]. According to this method, acetylthiocholine (AcSCH) is hydrolyzed by AChE to acetic acid and thiocholine. The catalytic activity is measured by the increase of the yellow anion, 5-thio-2-nitrobenzoate, produced from thiocholine when it reacts with 5,50-dithio-bis-2- nitrobenzoic acid (DTNB). AChE activity was expressed in μmol of AcSCH hydrolyzed/ min/ mg protein.

1.3 Lipid peroxidation

Serum malondialdehyde, a product of lipid peroxidation, was measured by a thiobarbituric reaction described by Kei Sathoh [(4)]. Serum proteins were precipitated by trichloro acetic acid (TCA) and the mixture was heated for 30 minutes with thiobarbituric acid in 2M sodium sulphate, in a boiling water bath. The resulting chromogen was extracted with n-butyl alcohol and the absorbance of the organic phase was determined at a wavelength of 530nm. The values were expressed in terms of nmol/ml of malondialdehyde (MDA) using 1,1,3,3, tetra ethoxy propane as the standard.

1.4 Superoxide dismutase

Activity of erythrocyte superoxide dismutase (SOD) was measured by the method of Marklund and Marklund [(5)]. Superoxide anion is involved in the auto oxidation of pyrogallol at alkaline pH 8.5. The superoxide dismutase inhibits the auto-oxidation of pyrogallol, which can be determined as an increase in absorbance per two minutes at 420nm. The SOD activity was measured as Units/gms of Hb. One unit of superoxide dismutase is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto oxidation.

1.5 Catalase

Catalase was measured by the method of Aebi 1983 [(6)]. Heparinized blood was centrifuged and the plasma was removed. The erythrocytes were washed three times with 5 ml 0.9% sodium chloride and lysed in 10 volumes of cold deionised water. The whole mixture was centrifuged further for 10min at 3,000 rpm. The cell debris was removed and the clear hemolysate was diluted 500 times phosphate buffer (60mM, pH- 7.4). Catalase decomposes hydrogen peroxide (H_2O_2) to form water and molecular oxygen. In the ultra violet range, H O shows a continual increase in absorbance with decreasing wavelength. At 240nm, H_2O_2 absorbs maximum light. When H_2O_2 is decomposed by catalase then the absorbance decreases. The decreased absorbance was measured at 240nm at 15 second intervals up to 1min and the difference in absorbance (Δ A at 240nm) per unit time was measured. The unit of catalase activity was expressed as mM of H_2O_2 decomposed/mg Hb/min.

RESULTS

Table 1 shows the incidence of poisoning in males and females. Out of the total 106 patients 80 were males and 36 were females. This shows that males constituted 68.96 % while as female constituted 31.03% of the total cases.

Table 1 Sex

Sex	No of cases	Percentage(%)
Males	80	68.96
Females	36	31.03

The distribution of the cases with respect to the cause of poisoning is shown in table 2. Out of total of 116 cases, 105 were found to be suicidal,6 cases were found to be accidental and 5 were found to be homicidal. This constituted 91.21, 6.97, 5.8 percentage respectively.

Table 2 Nature of poisoning cases

Nature of poisoning	No. Of cases	Percentage
Suicidal	105	91.21
Accidental	6	6.97
Homicidal	5	5.8

As far as the geographic distributions of the patients were concerned, 96 patients were found to be from rural area and 20 patients were from urban area. Thus 83.01% were rural residents and 17.24% were urban residents.

Table 3. Geographic distribution

Distribution	No.of cases	percentage
Rural	96	83.01
Urban	20	17.24

Table 4 shows AChE activity (mean ± SD) in erythrocytes in the control and various patient groups

Table 4

Group	µmol of AcSCH hydrolyzed/ min/ mg protein
Control	1.29 ± 0.13
Grade 1	0.91 ± 0.03*
Grade 2	0.68 ± 0.10*
Grade 3	0.54 ± 0.04*
Grade 4	0.44 ± 0.01*
Grade 5	0.38 ± 0.02*

Table 5

Group	Serum MDA (nmol/ml)
Control	4.23±0.008
Grade 1	4.53±0.00
Grade 2	4.89±0.016
Grade 3	5.87±0.273*
Grade 4	5.97±0.20*
Grade 5	6.01± 0.12*

Table 5 shows organophosphorus poisoning induced lipid peroxidation.

Table 6 shows superoxide dismutase and catase activity (Mean±S.D) in control and various groups

Table 6

Groups	Superoxide dismutase (U/gmHb)	Catalase (Mm H2O2 decomposed/mgHb/min)
Control	12.54±0.0007	15.88±0.198
Grade 1	17.07± 0.139	17.86 ±0.123
Grade 2	19.69± 0.008	19.69±0.008
Grade 3	9.86±0.126	11.021±0.007
Grade 4	8.36± 0.24	10.04±0.01
Grade 5	3.99±0.113	9.97± 0.62

DISCUSSION

On analysing the demographic profile our study found that the incidence of poisoning was more common among males than females. We found most cases belonged to rural areas. This could be attributed to more wide spread use and easy availability of Ops in rural areas. These results are similar to those found by Mitesh D. Falia [(7)].

The study was primarily focused to elucidate the role of free radicals in organophosphorus toxicity.

These compounds manifest their toxicity by irreversibly inhibiting the enzyme acetylcholinesterase (AChE) at the nerve synapse resulting in excessive accumulation of ACh, leading to the paralysis of cholinergic transmission in the CNS, autonomic ganglia, parasympathetic nerve endings, some sympathetic nerve endings and neuromuscular junction [(8)]. In our study the activity of acetylcholine esterase was significantly inhibited at all grades (Table 2) with maximum inhibition found in the most seriously affected patients. Similar results were obtained by Veerappa et al [(9)]. Inhibition of AChE appears to be the principal mode of action of organophosphorus compounds [(10)].

It is reported that besides their inhibitory effect on AChE, they also induce changes characteristic of oxidative stress [(11)].

Superoxide dismutase (SOD is a free radical (superoxide anion; O_2^-) scavenger. In

the present study, the activity of superoxide dismutase was increased at grade 1 and 2 as compared to control. The increased activity of SOD reflects an activation of the compensatory mechanism through the effects of pesticides on progenitor cells, and its extent depends on the magnitude of the oxidative stress and hence, on the dose of stressor [(12)]. Superoxide dismutase activity was recorded to be decreased at grade 3, 4 & 5. The efforts of the endogenous antioxidant enzymes to remove the continuously generated free radicals initially increase due to their induction but later enzyme depletion results [(13)].

Further, Superoxide Dismutase catalyzes dismutation reactions resulting in the generation of hydrogen peroxide (H_2O_2) from free radicals. This H_2O_2 is decomposed to water and molecular oxygen by the action of enzyme In the present study, the activity of Catalase was found to show trends similar to those of SOD. Its activity as compared to the controls, was increased at grade 1 and 2 while it was reduced at grade 3, 4 & 5.

The elevated activity of SOD & catalase found in our study is thus suggested to be due to adaptive response to the generated free radicals [(14)].

When the free radical production overwhelms the endogenous antioxidant levels, all the major biomolecules like lipids, proteins, and nucleic acids may be attacked by free radicals, but lipids are probably the most susceptible. [(15)] The oxidative destruction of lipids (lipid peroxidation) is a destructive, self-perpetuating chain reaction, releasing malonyl aldehyde (MDA) as the end product [(13)]. Our study detected an elevation in MDA indicating enhanced lipid peroxidation. The lipid peroxidation was significantly increased at grade 3, 4 and 5.It is suggested that organophosphorus induced lipid peroxidation at higher grade could possibly result from an enhanced microsomal oxidative capacity induced by OP. Ranjbar et al. reported significant lipid peroxidation accompanied with decreased AChE activity [(16)].

From our study we conclude that though the effects of Organophosphorus poisoning may be primarily ascribed to the fall in the activity of Acetylcholine esterase activity, there are significant but subtle changes in the cellular Oxidative system resulting in more widespread, as yet, un-quantified cellular damage. Further research towards the precise estimation of such cellular destruction and any proposed treatment directed towards such damage may help us to augment the current standard of care treatment resulting in better prognosis of OP poisoning.

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