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Oxidative Stress Markers in Patients with Organophosphorus Poisoning

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Abstract

Suicide is the second most common cause of death in the age group of 21-30 years. The organophosphorus poisoning is the common cause of suicide in this age group. Organophosphorus [OP] compounds have been widely used for a few decades in agriculture for crop protection and pest control. Some OP compounds are also used in the medical treatment. Organophosphate binds with cholinesterase enzyme and inhibits their activities by irreversible phosphorylation. This results in high levels of acetylcholine thus stimulating the muscarinic and nicotinic receptors resulting in consequent toxicity. The complications of OP poisoning includes acidosis, respiratory paralysis, acute renal failure, seizures, arrhythmia, aspiration etc and death may be due to combination of one or above complications. OPs may produce oxidative stress in different tissues through formation of reactive oxygen species (ROS). All the major biomolecules such lipids, proteins, and nucleic acids may be attacked by free radicals (ROS), but lipids are probably the most susceptible. We have estimated the serum malondialdehyde (MDA) as a lipid peroxidation product and erythrocyte antioxidant enzyme SOD in organophosphorus poisoning patients and healthy controls. We found significantly increased serum MDA and erythrocyte SOD in OP poisoning patients as compared to controls ($p<0.001$). OP compounds may induce oxidative damage by elevating lipid peroxide levels. Elevated activity of antioxidant enzyme may be attributed to adaptive response of erythrocytes to oxidative damage due to OP poisoning.

Keywords: Organophosphorus poisoning, Malondialdehyde, Oxidative damage

INTRODUCTION:

According to the 2012 WHO report, suicide is the second most common cause of death in the age group of 21-30 years. The organophosphorus poisoning is the common cause of suicide in this age group (1). Organophosphorus [OP] compounds have been widely used for a few decades in agriculture for crop protection and pest control. Some OP compounds are also used in the medical treatment (2).

Organophosphate binds with cholinesterase enzyme and inhibits their activities by irreversible phosphorylation. This results in high levels of acetylcholine thus stimulating the muscarinic and nicotinic receptors resulting in consequent toxicity (3). The complications of OP poisoning includes acidosis, respiratory paralysis, acute renal failure, seizures, arrhythmia, aspiration etc and death may be due to combination of one or above complications (2).

OPs may produce oxidative stress in different tissues through formation of reactive oxygen species (ROS) (4, 5). All the major biomolecules such lipids, proteins, and nucleic acids may be attacked by free radicals (ROS), but lipids are probably the most susceptible. The oxidative destruction of lipids is known as lipid peroxidation and malondialdehyde (MDA) as the end product of lipid peroxidation (5, 6). Once formed, these free radicals initiate their own reactions thereby exerting potentially harmful effects on various systems of the body. Normally these ROS are converted to less reactive compounds by the use of antioxidant. In normal cell, there are appropriate pro-oxidants (free radicals): antioxidant balance. However, this balance can be shifted toward the pro-oxidants when production of oxygen species is increased greatly (e.g. following ingestion of certain chemicals or drugs) or when levels of antioxidants are diminished (e.g. by inactivation of enzyme involved in disposal of oxygen species and by conditions that cause low levels of antioxidants). This is called as oxidative stress. When oxidative stress is massive or prolonged that can result in serious cell damage (6).

Abdulaziz M. Al-Othman etal explained the mutagenic activity of OP compound (malathion). This may be due to the presence of electrophilic sites in it or its metabolic intermediates. They found oxidative damage in rat liver when exposed to malathion . Liver is a primary site of oxidative metabolism and biotransformation by using cytochrome P450 activity (4).

The present study was planned to examine alteration in the level of MDA and SOD in organophosphorus poisoning patients.

MATERIALS AND METHODS:

The present study was carried out in the Department of Biochemistry, Government Medical College and Hospital, Miraj (Maharashtra, India). Study protocol was approved by ethical committee, Government Medical College. Miraj.

Sample size: The study group included total 80 subjects. This includes patients as well as control.

Patients: Total 40 patients with organophosphorus poisoning hospitalized at Government Medical College and Hospital. The diagnosis of the patient was done by the clinicians on the basis of the patient's condition, smell of the Organophosphorus poisoning compound, clinical history, personal history, physical examination.

Control: The 40 healthy controls were taken in all age group with both genders attending the OPD of Government Medical College and Hospital, Miraj during the same period. Previous history of accidental or suicidal poisoning and no any abnormal clinical findings, particularly in the context of metabolic and nutritional disorders were excluded from the study.

Blood Collection: Informed consent was obtained from the participants. 1 ml blood was collected in plain bulb and 1ml blood was collected in bulb having anticoagulant (heparin) from the patients and control under aseptic condition by venipuncture using 2 ml sterile disposable syringe and needle. Blood samples from plain blub clear serum was separated and used for estimation of MDA. Blood samples from heparin bulb were centrifuged and plasma was removed. Erythrocytes were washed with normal saline for three times and used for estimation of SOD level.

Serum MDA level was measured by thiobarbituric acid reaction described by Kai Satoh (7) and the levels were expressed as nmol/ml. Superoxide dismutase activity was estimated in the erythrocyte by method described by Marklund and Marklund (8) and levels was expressed as u/ml. The data were evaluated statistically by using student't' and 'F' test, 'F' value was calculated by Minitab and SPSS software.

RESULTS

Table no 1 shows serum MDA and erythrocyte SOD levels in subjects. We found significantly increased serum malondialdehyde (MDA) and superoxide Dismutase (SOD) levels in OP poisoning patients as compared to control.

Table No.1. Serum MDA and erythrocyte SOD levels in subjects

Parameters	Patients(n=40) (Mean ± SD)	Controls (n=40) (Mean ± SD)
Serum Malondialdehyde (nM/ML)	5.20* ± 0.98	2.67 ± 0.88
Superoxide Dismutase (U/GM)	15.94* ± 1.28	12.76 ± 1.64

*p<0.001, Highly Significant

Table 2 shows the variation in serum MDA and SOD levels in subjects with respect the different age groups. In the age group 21 – 40 and 41- 60 years, we found significant difference in MDA and SOD. Whereas in age group upto 20 and above60 years non-significant difference in MDA and SOD.

Table No. 2: Serum MDA and erythrocyte SOD levels in subjects with respect to age

	Patients			Controls		
Age group (In years)	N	MDA (nM/ml)	SOD (U/gm)	N	MDA (nM/ml)	SOD (U/gm)
Up to 20	04	$6.15^{NS} \pm 0.81$	$15.21^{NS} \pm 0.94$	02	3.1 ± 1.0	12.06 ± 1.18
21 to 40	23	$5.26^{*} \pm 0.76$	$15.91^{*} \pm 1.29$	17	2.65 ± 0.91	13.09 ± 1.46
41 to 60	09	$4.76^{*} \pm 1.16$	$16.21^{*} \pm 1.26$	16	2.64 ± 0.71	12.68 ± 1.62
Above 60	04	$5.00^{NS} \pm 1.28$	$16.15^{NS} \pm 1.64$	05	2.50 ± 1.24	12.22 ± 2.33

* $p<0.001$, Highly Significant, NS= Non significant

Table No. 3 shows serum MDA and erythrocyte SOD levels in subjects with respect to sex. We found non-significant difference in these levels.

Table No. 3: Serum MDA and erythrocyte SOD levels in subjects with respect to sex

Sex	Patients			Control		
	N	MDA (nM/ml)	SOD (U/gm)	N	MDA (nM/ml)	SOD (U/gm)
Male	25	5.06 ± 1.05	15.83 ± 1.08	19	2.30 ± 0.74	13.13 ± 1.4
Female	15	5.44 ± 0.80	16.11 ± 1.56	21	2.99 ± 0.88	12.42 ± 1.72
Significance	t p	-1.28 0.209	-0.60 0.549	t p	-2.66 0.011	1.40 0.170

DISCUSSION

We estimated the levels of MDA and SOD in OP poisoning patients and healthy controls and are given in table no. 1. The mean levels of serum MDA and erythrocyte SOD were increased significantly in patients as compared to control ($p<0.001$). This may indicate oxidative stress in OP poisoning patients. Toxic effects induced by OP compounds may be associated with increased generation of reactive oxygen species (ROS) which induces the oxidative process and lipid peroxidative damage in cell membranes. Increased production of ROS and enhanced lipid peroxidation are considered responsible for the toxicity due to pesticides (9).

The inhibition of ChE initiates the accumulation of free radicals leading to lipid peroxidation, which may be the indicator of cell injury (10). Similarly previous study showed increased serum MDA and erythrocyte SOD in OP poisoning patients (5, 9, 10)

SOD is the first line of free-radical clearance in tissues and red blood cells and it decomposes superoxide anionic free radicals (11). Under normal physiological conditions, the enzymatic and non-enzymatic antioxidants in the body can clear the free radicals produced in metabolic processes and maintain a dynamic equilibrium between oxidation and antioxidants. Organophosphorus compounds may induce hypoxia, under hypoxic conditions, mitochondrial aerobic metabolism is down-regulated and the production of adenosine triphosphate decreases (11).

The present findings indicate that cells continually suffer from oxidative stress in spite of over activity of antioxidant defense mechanism as indicated by increase in erythrocyte SOD activity. The severity of OP poisoning shows more stress and hence more free radical generation. The free radical production is so high that it even overwhelms the elevated antioxidant failing to check lipid peroxidation. The higher levels of antioxidant enzymes may be necessary to detoxify increased concentration of lipid peroxidation products that are generated from oxidative stress due to OP toxicity (9).

Generally there is an inverse relationship between lipid peroxidation and antioxidant enzymes; however we found significant increase in serum MDA as well as erythrocyte SOD activity (9, 10). Erythrocyte SOD other antioxidant enzymes efficiently scavenge toxic free radicals and are partly responsible for protection against lipid peroxidation due to acute/chronic organophosphorus pesticide poisoning. Thus the increase in erythrocyte SOD was maybe a response towards increased ROS generation in OP poisoning (9).

CONCLUSION

OP compounds may induce oxidative damage by elevating lipid peroxide levels. Elevated activity of antioxidant enzyme may be attributed to adaptive response of erythrocytes to oxidative damage due to OP poisoning.

REFERENCES

[1] Dayanand Raddi and Anikethana G V. Liver Enzymes for Assessment of Severity of Organophosphorus Poisoning. International Journal of Medical and Health Sciences, Jan 2015; 4(1):60-63.

[2] K. N. Pujari, N.E. Pore, S. P. Jadkar. Serum Enzymes in Organophosphorous Poisoning. Journal of Medical Science and Clinical Research, June 2016; 4(6):10771-10778.

[3] Chidiebere Uchendu, Suleiman F. Ambali and Joseph O. Ayo. The organophosphate, chlorpyrifos, oxidative stress and the role of some antioxidants: A review. African Journal of Agricultural Research, May 2012; 7(18):2720-2728.

[4] Abdulaziz M. Al-Othman, Khaled S. Al-Numair, Gaber E. El-Desoky,

Kareem Yusuf, Zeid A. Al Othman, Mourad A. M. Aboul-Soud and John P. Giesy. Protection of α-tocopherol and selenium against acute effects of malathion on liver and kidney of rats. African Journal of Pharmacy and Pharmacology, Sept 2011; 5(10):1263-1271.

[5] J. Vidyasagar, N. Karunakar, M. S. Reddy, K. Rajnarayana, T. Surender and D. R. Krishna. Oxidative stress and antioxidant status in acute organophosphorous insecticide poisoning. Indian Journal of Pharmacology, April 2004; 36(2):76-79.

[6] K. N. Pujari and S.P. Jadkar. Superoxide dismutase levels in leukemias. International Journal of Basic Medical Sciences May 2011, 2(2):96-100.

[7] Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica. Chimica Acta 1978; 90: 37-43.

[8] Marklund and Marklund. A simple assay for super oxide dismutase using autooxidation of pyrogallol. Eur. J. Biochem. 1974; 47:469-74.

[9] Hundekari IA, Suryakar AN and Rathi DB. Acute organo-phosphorus pesticide poisoning in North Karnataka, India: oxidative damage, haemoglobin level and total leukocyte. African Health Sciences 2013; 13(1): 129 – 136.

[10] Indira A Hundekari, A N Suryakar, and D B Rathi. Oxidative stress and antioxidant status in acute organophosphorous pesticides poisoning cases of North Karnataka (India). Journal of Environmental Health Research 2011; 11(1):39-44.

[11] JW Zhang, GC Lv and Y Zhad. The Significance of the Measurement of Serum Xanthine Oxidase and Oxidation Markers in Patients with Acute Organophosphorus Pesticide Poisoning. The Journal of International Medical Research 2010; 38: 458 – 465.