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Antioxidant status and oxidative stress in organophosphate pesticide poisoning

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Abstract: Objective: *To study the antioxidant status and the extent of oxidative stress in patients with organophosphate poisoning before and after specific treatment.*
Material and methods *: The study was conducted in 50 OP poisoned patients. Superoxide dimutase (SOD), catalase (CAT) and malonalaldehyde levels were estimated as an index of antioxidant status and oxidative stress respectively and comparisons were made(a) healthy control subjects& sprayer poisoned patients(b) between pretreated &post treated patients, after specific treatment .*
Results *: There were significantly increase in superoxide dismutase(SOD)& catalase in the exposed sprayer groups, comparative to normal healthy control subjects .The increase in lipid peroxidation as reflected by elevated levels of malonalaldehyde (MDA) in the pesticide exposed group, indicates oxidative stress. There were progressive improvement observed in the both SOD&CAT levels after specific therapy in post treated patients, comparative to pretreated patients. Significant compensatory level of malonalaldehyde(MDA)were observed in post treated patients in comparison to pretreated patients. The level of improvement in post treated patients also depends on the severity grade of poisoning. .*
Conclusion*: The increased level of MDA in OPP patients who failed to survive was probably reflective of accelerated lipid peroxidation, cell damage &death(oxidative stress).significant improvement was noticed in the SOD&CAT levels with specific treatment (atropine plus pralidoxime (PAM) therapy).*
Key Words *: Antioxidant status ,free radicals ,malonalaldehyde (MDA) ,pesticide poisoning ,superoxide dismutase(SOD) ,catalase(CAT) .*

I. Introduction

Pesticides are biocides capable of killing all forms of life. They are some of the deadliest; poisons produced by man, hence present a health hazards in long term exposure even at low levels. Organophosphates (OP) pesticides are commonly used worldwide in agricultural and in pest control.

Clinical manifestations of OP pesticides poisoning are caused by excessive synaptic accumulation of acetylcholine (ACh)[1] . Organophosphate compounds irreversibly inhibit the enzyme acetyl cholinesterase (AChE) and butyryl cholinesterase(BuChE), 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[2] .BuChE inhibition , by contrast appears not to result in clinical features ,however its activity is more easily measured than AChE activity and BuChE assays are widely available and therefore commonly recommended in the early assessment of OP pesticide poisoned patients.[3]
It is reported that OP pesticides, besides there inhibitory effect on AChE ,also changes characteristic of oxidative stress [4].Superoxide dismutase (SOD) ,whose substrate is free radical (superoxide anion; O2.-) catalyzes dismutation reaction resulting in the generation of hydrogen peroxide. This H_2O_2 is decomposed to water and molecular oxygen by the action of catalase .When the radical production overwhelms the endogenous levels decreased , they cause considerable cell damage / death .All the major biomolecules such as lipid protein and nucleic acids may be attacked by free radicals, but lipids are probably most susceptible .[5]
The cells have different mechanism to alleviate and repaired damaged macromolecules .The primary defense is offered by enzymatic and non enzymatic antioxidants which have been shown to scavenge free radicals and reactive oxygen species (ROS).The antioxidant enzymes ,SOD,CAT and glutathioneperoxidase (GPX) have been shown to be significantly affected by pesticides[6]. Antioxidants belonging to the second line of defense including glutathione(GSH), vitamin C, Vitamin E(mainly α-tocopherol) and β carotene.[7]
O.P. poisoning may induce oxidative stress leading to generation of free radicals. Free radicals are formed which then increases oxidative destruction of lipids (lipid peroxidation), is a destructive self perpetuating chain reaction, releasing malonylaldehyde(MDA) as the end product.[8]

In view of this our study included antioxidant parameters such as superoxidedismutase, catalase and oxidative stress lipid peroxides(malonylaldehyde).

II. Material and Methods

The present study was conducted at the MGM Medical College, Navi Mumbai, during the period of Jan'08 to Dec'09. Informed consent was obtained from the pesticide sprayers. The study was cleared by The University Ethics Committee, MGM University of Health Sciences, Navi Mumbai. Fifty male pesticide sprayers were involved in the age group of 17-55 years engaged in pesticide handling and spray. They were several years exposed to pesticides through accidental inhalation during the spraying. This study was compared with 50 normal healthy control subjects.

Collection of samples

Blood samples were collected in a total 50 O.P. pesticides patients along with 18 normal healthy subjects as a control. In 10 cases samples were collected prior to and 24 h after treatment with atropine plus PAM. 10 ml of venous blood was collected from each subject in sterile, heparin (200 units)containing vials. The blood was immediately centrifused at 3000 rpm for 15 minute and the plasma separated. The cells were washed with normal saline and RBC's were subjected to lysis.

Lipid peroxides estimation[9]

The product of lipid peroxidation Malondialdehyde was estimated in the blood by the method Stocks and Dormandy,1971. 0.5 ml of blood in phosphate buffer (pH 7.4; 0.1 M) was incubated for 30 minutes at 37^0 c and centrifuged. To the supernatant (3 ml) collected added to 1 ml, 1% TBA and then placed in boiling water bath for 15 minutes. Contents were cooled in ice water and centrifuged for 15 minutes at 2500 rpm. The absorbance was taken against a suitable blank at 532 nm and was converted to equivalent of MDA (malondialdehyde) (nmol/ ml blood) using molar extinction coefficient of 1.56×10^5 mole/$L^{-1}cm^{-1}$.

Catalase estimation[10]

CAT activity was determined by the method of Sinha (1979), using H202 as substrate and expressed as µmol H2O2 decomposed/min/g Hb The isolated blood corpuscles were washed twice with 0.9% NaCl solution. They were then lysed with 20 parts of cold water. The lysate was used as such or the hemoglobins were removed beforehand.. The lysate was properly diluted with water for the assay of its catalase content.

Superoxide dismutase estimation[11]

Superoxide dismutase (SOD) activity determination, we adopted the method of Misra and Fridovich (1972).Two ml of packed cells were lysed by addition of an equal volume of cold deionized water. Hemoglobin was then precipitated by adding chloroform: ethanol (1.5: 1) mixture. The mixture was centrifuged at 3000 rpm for 15 min, and the SOD activity was measure in the supernatant. To 0.88 ml of riboflavin solution (1.3×10^{-5}mM in 0.01M potassium phosphate buffer, pH 7.5) 60 µl of O-dianisidine solution (10^{-2} mM in ethanol) was added. To this 1 ml of distilled water was added and kept away from light. Hundred µl of the separated SOD was added and optical density (OD) measured at 460 nm using the spectrophotometer. The cuvette was against blank containing ethanol in place of enzyme. The SOD was estimated from the standard graph plotted using different concentrations of pure bovine then transferred to the illumination box for exactly 4 min and the OD was remeasured against blank containing ethanol in place of enzyme.The SOD was estimated from the standard graph plotted using different concentrations of pure bovine SOD .

III. Result:

Table-1

Biochemical Profile of Pesticide Sprayers Compared to Controls

Bio chemical Parameters	Controls(n=18) Mean ±S.D.	Sprayers (n=50) Mean ±S.D
MDA (nmol/TBARS/ml blood)	9.43 ±0.78	26.30 ±2.02*
CAT (µ molH_2O_2hydrolysed $\times 10^4$/ml/g Hb)	105.5± 15.20	135.69 ± 12.34
SOD (µ mol hydrolysed $\times 10^4$/ml/g Hb)	3.29 ± 0.79	8.03 ± 2.46

Statistically *Significant p<0.05*

Table-2
Pre and post treated biochemical profile of acute pesticide poisoning cases(n=10)

Biochemical parameters	Pre-treated blood sample mean ± S.D	Post treated blood sample mean ± S.D
MDA n mol/ TBARS/ml blood	**36.20 ± 2.02**	**13.60 ± 3.46**
CAT (µmolH_2O_2hydrolysed X 10^4/ml/g Hb)	**130.5 ± 31.6**	**61.9 ± 20.6**
SOD (µ molhydrolysed X 10^4/ml/g Hb)	**7.89 ± 2.41**	**3.69 ± 2.67**

MDA

The mean ± SD blood level of MDA was 26.30±2.02 nmol/TBARS/ml, which was significantly higher than their healthy control(9.43±0.78). The increase in lipid peroxidation as reflected by elevated levels of MDA in pesticide exposed group indicates oxidative stress(table-1).

The mean ± SD blood level of MDA in post treated blood sample was **13.60 ± 3.46** nmol/TBARS/ml, which was significantly lower than their pre treated blood sample(**36.20 ± 2.02)**. The decreased MDA level ,reflected the recovery in lipid peroxidation.

CAT

The mean ± SD blood level of CAT in exposed subjects was 135.69±12.34(µmol H_2O_2 hydrolysed x10^4 /ml/g Hb),while it was105.5±15.20 in respective healthy control. It showed elevated CAT activity in this study, as a result of OP pesticide induced poisoning .The increased activity of CAT seen in the poisoning cases coupled with an increase in the blood lipid peroxidation level (MDA) suggest an insufficient antioxidant defense .

The mean ± SD blood level of CAT in post treated blood sample was **61.9 ± 20.6,**while it was**130.5 ± 31.6** pretreated blood sample, suggest the primary action of pralidoxim is to prevent inhibition of the enzyme activity.

SOD

The mean ±SD blood level of SOD value were 8.03 ± 2.46 (µ mol hydrolysed X 10^4 /ml/g/Hb) in exposed sprayers as compare to normal healthy control(3.29 ±0.79). Exposure to OP pesticide showed elevated SOD activity in this study, SOD effectively dismutases superoxide anion into hydrogen peroxide and O_2.

The mean ±SD blood level of SOD in post treated sample was 3.69±2.67 (µ molH2O2hydrolysed X 104/ml/g Hb) , while it was 7.89±2.41 in pretreated blood sample again sggest the primary action of pralidoxime is to prevent inhibition of the enzyme activity.

IV. Discussion:

OP pesticide poisoning is primarily a problem of developing countries like India. In the present study, it was found that increased neurological symptoms count were observed among the pesticide sprayers and farmers suffered from acute OP pesticide poisoning due to either occupational or intentional inhalation or ingestion of OP pesticides. Our findings support the previous studies of OP pesticides and neurological symptoms reported in farm workers (Gomes et al 1998; strong et al 2004)[12], green house workers (Walcark et al 1999)[13] and factory workers (Bellin and Chew 1974)[14.]

The role of oxygen free radical (OFR)has been well established in many chronic disorders. The signification of the implication of OFR in acute condition like OP pesticide poising has not been investigated so far .

The effects of the organophosphates on fish revealed that besides AChE inhibition ,There were changes characteristic of oxidative stress[15] . In humans OPIs were shown to reduce the total cholesterol and phospholipids level of RBC membrane following Phosphamidon and malathion[16] .The basis of OPI toxicity in the production of OFR may be due to –

1) Their "redox –cycling" activity –they readily accept an electron to farm free radicals and then transfer them to oxygen to generate superoxide anions and hence hydrogen peroxide through dismutation reaction[17.]

2) Generation of free radicals probably because of the alteration in the normal homeostasis of the body resulting in oxidative stress ,if the requirement of continuous antioxidants is not maintained .

In the present study, it was observed that the antioxidant enzymes, superoxide dismutase (SOD)and Catalase (CAT) in untreated pesticide sprayers were significantly affected. The acute poisoning in sprayers lead to elevated MDA level as well as increased activities of catalase (CAT) and SOD. Prakasam et al (2001)[18] also reported significant elevation of MDA levels in pesticides sprayers' thereby suggesting oxidative stress (Bachowski et al 1998). Vidyasagar et al (2004) also found higher MDA level with inhibited AChE activity of OP poisoned patients. The antioxidant system has been found to be susceptible to damage by OP pesticides

(Kale 1999) and these pesticides have potential to generate free radicals in biological system (Hazarika et al 2003), have reported that oxidative stress induces an efflux of GSSG from erythrocytes, which may decreases the red blood cell GSH. It is possible that, the decreased GSH level observed in this study was not sufficient to combat with the enhanced production of MDA during increased lipid-peroxidation as a result of pesticides poisoning (Yoshioka et al 1997)[19]. It has also been advocated that the cysteinyl residue of GSH offers a nucleophilic thiol which is important in the detoxification of electrophilic metabolites and metabolically produced oxidizing agents.

GSH is a substrate of enzymes namely GPx and Glutathione-S- transferase. GSH/GSSG ratio is a very important indicator of the redox status of the cell (Rana et al 2002)[24]. It has been observed that pesticides poisoning disturb this ratio, again confirming the presence of oxidative stress). Our results suggest that pesticide poisoning induces oxidative stress by depleting intracellular GSH and increasing ROS production. GSH is known to play a key role in regulating intracellular levels of ROS by scavenging free radicals to maintain the intracellular redox status. Organophosphate pesticides appear to disturb this key cellular pathway perhaps by disrupting mitochondria metabolism as suggested recently by Oelgado et al 2006; Chan et al 2006 and Sherer et al. [25]

The increased activity of CAT seen in the poisoning cases coupled with an increase in the lipid peroxidation level (MDA) suggests an insufficient antioxidant defense. CAT, an enzyme that transforms hydrogen peroxide into hydrogen and oxygen, plays an antioxidant role and its activity increasing in acute poisoning submitted to oxidative stress. SOD has been reported to participate in the reduction of superoxide anion to less reactive molecular oxygen and hydrogen peroxide species. The H_2O_2 can be further decomposed by CAT or by glutathione peroxidase in the presence of reduced glutathione. Compound with other antioxidative enzyme, SOD and CAT are more sensitive and efficient and respond more rapidly in the face of oxidative stress (Dewez D et al 2005)

The pharmacological treatment of OP poisoning is based on anticholinergic and anticonvulsant drugs as well as oximes. In 10 cases suffering from acute OP poisoning treatment with oxime (Pralidoxime chloride (2-PAM), a monopyridinium was administered in a dose of 500mg/h, continuously maintained until clinical improvement was obtained or full recovery occurred. As per IPCS (1989), the therapy can continue for 18 hours or longer, depending on the clinical status of an individual case of OP pesticides poisoning. An initial trial dose of atropine as antimuscarinic agent (0.05mg/kg) was also given intravenously and then repeated every 10-15 min. Atropine was repeated till patient is atropinized (dilated pupils, dry skin and skin flushing).

The post treatment blood samples were drawn and measures of biochemical parameters such as MDA, GSH, CAT and SOD were done in each of the 10 cases selected.

Following treatment with a combination of pralidoxime and Atropine the thiobarbituric acid reactive substances (TBARS), a measure of lipid peroxidation (MDA) level decreased significantly as well as activity of SOD and CAT were also significantly inhibited thereby indicating attenuation of oxidative stress imposed by OP pesticide poisoning. Generation of oxidative stress and consequent lipid peroxidation by OP pesticides could be due to high concentration of polyunsaturated fatty acids in the cells, lipid peroxidation is a major outcome of the free radicals mediated injury. Two broad outcomes of lipid peroxidation are structural damage of cellular membranes and generation of oxidized products, some of which are chemically reactive and may covalently modify cellular macromolecules. These reactive products are thought to be the major effecter of tissue damage from lipid peroxidation(Mattison,1998) It is concluded that there was a considerable increase in lipid peroxide levels indicating an enormous oxidative stress in OP pesticide sprayers. Elevated levels of CAT&SOD in OP pesticide sprayers suggest that insufficient antioxidant defense . Significant improvement was noticed in post treated patients with compensatory alterations in antioxidant statusand oxidative stress..

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References:

[1]. Senanayake N, Johnson R. Acute polyneuropathy after poisoning by a yew organophosphate insecticide. N Engl J Med 1992;302:155-7.
[2]. Vishwananthan R. Srinivasan V. Treatment of OP compound poisoning J Indian Med Assoc 1964:43:494-7
[3]. Namba T, Nolte C, Jackrel J, Grob D. Poisonong due to organophosphate insecticides. Am J Med 1971; 50: 475-92.
[4]. Malkovics B. Effect of organophosphates on the antioxidant systems of fish tissue. Acta Biol Hung 1995;9;11
[5]. A. Gupta, A. Gupta, G. S. Shukla ,Effects of neonatal quinalphos exposure and subsequent withdrawal on free radical generation and antioxidative defenses in developing rat brain ,J.Appl.Toxicol.18(1998)71-77.
[6]. F.N .Bebe ,M.Panemangalore,Pesticides and essential minerals modify endogenous antioxidants and cytochrome P_{450} in tissues of rats ,J.Environ.Sci.Health B40(20005)(769-784)
[7]. M.Irshad ,P.S.Chaudhuri,oxidant-antioxidant system.:role and significance in human body,Indian J.Exp.Biol.40(2002)(1233-1239

[8]. Cheeseman KH. Lipid peroxidation in biological system. In Halliwel B, Aruoma OK, editors, DNA and Free radicals, London: Ellis Horwood; 1993, p.201-11
[9]. Stocks J, Dormandy TL. Auto-oxidation of red cell lipids induced by hydrogen peroxide. Br J Haematol 1971; 20: 95-111.
[10]. Sinha AK. Colorimetric assay of catalase. Anol Biochem 1979;47:389–94.
[11]. Misra HP, Fridovich E. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem1972;247(10):3170–5.
[12]. Gomes J, Lloyd O, Revitt MD, Basha M. Morbidity among farm workers in a deseart country in relation to long term exposure of pesticides. Scand J Work Environ Health, 1998; 24: 213-219.
[13]. Bazylewicz- Walcazak B, Majczakowa W, Szymczak M. Behavioral effects of occupational exposure to organophosphate pesticides in female greenhouse planting workers. Neurotoxicology, 1999; 20:819-826.
[14]. Bellin J, Chow I. Biochemical effects of chronic low level exposure to pesticides. Res Comm Chem Pathol Pharmacol, 1974, 9:325-337.
[15]. Malkovics B. Effect of organophosphates on the antioxidant systems of fish tissue. Acta Biol Hung 1995;9;11
[16]. John S, Kale M, Rathore N, Bhatnagar D. Protective effects of Vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. J Nytr Biochem 2001; 12: 500-4
[17]. Ryrfeldt A, Bennenberg G, Moideus P. Free radicals and lung disease. In: Cheeseman KH, Slater TF, editors, Free radicals in medicine. London: Church Hill Livingstone; 1992. p. 588-603
[18]. Prakasam A, sethupathy S, Lalitha S. Plasma and RBC antioxidant status in occupational male pesticide sprayers. Clinica chemical Acta,2001; 310:107-112.
[19]. Yoshioka T, Lwamoto N, Tsunoda Y, hiquchi C, Kawaguchi HK, Ito K. Down regulation of manganese- superoxide dismutase gene expression in idiopatic nephritic syndrome . J Pediatr 1997; 130(5):800-807.
[20]. Yoshioka T, Lwamoto N, Tsunoda Y, hiquchi C, Kawaguchi HK, Ito K. Down regulation of manganese- superoxide dismutase gene expression in idiopatic nephritic syndrome . J Pediatr 1997; 130(5):800-807.
[21]. Delgado Eduardo HB, Streck EL, Quevedo JL, Dal-Pizzol F. Mitochondrial respiratory dysfunction and oxidative stress after chronic malathion exposure. Neurochemistry Re, 2006; 1021-1025.