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Original Research Article

OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN AGRICULTURAL PESTICIDE SPRAYERS

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ABSTRACT

Background: In India Agriculture is the principle occupation and considerable proportion of population, farmers, females and children are exposed to harmful effects of organophosphorous pesticides, it is reported that OPIs, besides their inhibitory effect on AChE, also induce changes characteristic of oxidative stress resulting in chronic diseases. In view of the possible oxidative stress involved in OPI poisoning, we have decided to estimate the levels of antioxidant status and oxidative stress in pesticide sprayers working in paddy fields by estimating blood levels of MDA, ADA, Vitamin C and SOD levels in blood.

Materials and Methods: The study was conducted at the Department of Biochemistry, Rangaraya Medical College with 62 farmers (study Group), and 30 (control group) volunteers from in and around Kakinada were participated in the study by giving consent form. Blood collected in EDTA and plain bottles. Serum was separated and analysis was done on the same day or stored at -80°c. The observed values are compared with control group for statistical analysis by using SPSS soft ware.

Results: In our present study MDA, ADA, and SOD levels are significantly elevated in chronic organophosphorous poisoning or sprayer population in compares with control group ($P < 0.001$, $P < 0.001$, and $P < 0.001$) respectively. The vitamin C level in the blood was significant decrease in sprayer population when compared to controls ($P < 0.001$), indicating low antioxidant status in study group.

Conclusion: Our results indicate that chronic, long term pesticide exposure will lead to change in antioxidant status and oxidative stress pattern which may result in chronic diseases.

KEY WORDS: Oxidative Stress, Antioxidant Status, Organophosphorous Insecticide, Pesticide, Sprayers.

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BACKGROUND

Seventy two percent of Indian population lives in rural areas where agriculture is the main source of income. Agriculture and allied activities account for 27% of the GDP, contribute to 21% of total exports earnings and provide livelihood to about 67% of the workforce [1]. Accordingly there is increased utilization of pesticides to increase the yield. However, only recently it has been postulated that longterm low exposure of these chemicals are increasingly linked to human health such as immunosuppression, endocrine disruption, reproductive abnormalities and cancer. Free radical damage and oxidative stress have been implicated as the basic cause for many chronic diseases. Organophosphorous

poisoning either chronic or acute changes of antioxidant status and causes oxidative stress as shown by many studies.

Organophosphorous compounds irreversibly inhibit the enzyme acetylcholinesterase (AChE), 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]. It is reported that OPIs, besides their inhibitory effect on AChE, also induce changes characteristic of oxidative stress [3]. Superoxide dismutase (SOD), whose substrate is a free radical (superoxide anion; O_2^-) catalyzes dismutation reaction resulting in the generation of hydrogen peroxide (H_2O_2). This H_2O_2 is decomposed to water and molecular oxygen by the action of catalase. When the free radical production overwhelms the endogenous antioxidant levels, they cause considerable cell damage/death. All the major biomolecules like lipids, proteins, and nucleic acids may be attacked by free radicals, but lipids are probably the most susceptible [4].

The oxidative destruction of lipids (lipid peroxidation) is a destructive, self-perpetuating chain reaction, releasing malonyl aldehyde (MDA) as the end product [5].

In view of the possible oxidative stress involved in OPI poisoning, we have decided to estimate the oxidative stress caused by chronic exposure of organophosphorous pesticides in a sprayer population by estimating blood levels of MDA, ADA, Vitamin C and SOD levels in blood.

MATERIALS AND METHODS

The study was conducted at the department of Biochemistry, Rangaraya medical college with 62 farmers (study Group), and 30 (control group) volunteers from villages in and around Kakinada, Andhrapradesh in 2004 to 2006. The study group consists of 62 farmers who have been involved mainly in spraying activities in the paddy fields for more than five years from nearby villages. Inclusion criteria in to the study group (sprayer population) are- age less than 40 years, no history of chronic diseases and working period as sprayer more than 5 years. The control group consisted of 30 volunteers from the same place, same age and did not have any organophosphorous exposure. Consent was obtained from both cases and controls. Permission was granted from institutional ethics committee. Blood collected in EDTA and plain bottles from sprayers directly in spray field. Serum was separated by standard methods and analysis was done same day or stored at -80°C. ADA was estimated by Galantio Giusti colorimetric method, MDA by thiobarbituric acid reaction [6], vitamin C by photometric method (7), and SOD by pyrogallal method [8]. The observed values are compared with control group with statistical analysis by analyzing student't' test and ANOVA (analysis of variance) by using SPSS software and p-value ≤ 0.01 is considered as statistically significant.

RESULTS

Table 1: MEAN ± SD values of parameters in control and sprayer cases.

Parameter	Control (n=30) Mean ± SD	Sprayer population (n=62) Mean ± SD
MDA (nmol/dl)	264 ± 45	342 ± 77
ADA (U/L)	7.5 ±0.5	16.2 ± 7.3
Vitamin C (mg/dl)	0.92 ± 0.18	0.66 ± 0.26
SOD (U/ml)	2.7 ± 0.65	6.1 ± 2.0

Table 2: Showing the 't' value and p value of control vs sprayer (chronic organophosphorous) cases.

Parameter	't' value	P value
MDA	6.11	< 0.001
ADA	9.34	< 0.001
VITAMIN C	5.56	< 0.001
SOD	12.13	< 0.001

DISCUSSION

In developing and agricultural country like India there is increased use of pesticides specially organophosphorous insecticides. With green revolution and industrialization, OPI became house hold items of agriculturists, resulting in chronic exposure while spraying in the fields, as well as to commit suicides because of their easy accessibility and availability. In our present study we took samples from 62 sprayers while they are in the fields. Almost all sprayers take no protective measures like wearing masks, gloves, clothing etc. They don't know the advantage of taking protective measures. Most of the sprayer population belongs to low socio-economic strata.

The role of oxygen free radicals (OFR) has been well established in many chronic disorders.

The effects of organophosphates on fish revealed that besides AChE inhibition, there were changes characteristic of oxidative stress [3]. In humans, OPIs were shown to reduce the total cholesterol and phospholipid level of RBC membrane following phosphamidon and malathion, and increase lipid peroxides level following malathion [9].

In our present study MDA levels are significantly elevated in chronic organophosphorous poisoning in comparision with control group ($P < 0.001$) as shown in table no 1 & 2 similar findings were found in the studies (10-14). Longterm pesticide exposure causes DNA damage, nucleic acid metabolism affected to some extant resulting elevated ADA levels in our present study. Elevation of ADA is significant in chronic exposure or sprayer population when compared to controls ($P <0.001$) as shown in table 1&2, indicating increased purine catabolism.

In the present study the antioxidant status of the individual was assessed by measuring the vitamin C level in the blood. There is significant decrease in vit C level ($P <0.001$) in sprayer population when compared to controls indicating low antioxidant status. SOD levels in blood increased significantly ($P <0.001$) in sprayer population when compared to controls in our study as shown in table no 1& 2. It may be due to the efforts of endogenous antioxidant enzyme to remove the continuosly generated free radicles. These findings are consistent with other studies [11-15].

The reason for OFR production in OPI toxicity may be

a) Their "redox-cycling" activity - they readily accept an electron to form free radicals and then transfer them to oxygen to generate superoxide anions and hence hydrogen peroxide through dismutation reaction [16].

b) 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.

The efforts of the endogenous antioxidant enzymes to remove the continuously generated free radicals initially increase due to an induction but later enzyme depletion results, resulting in oxidative cell damage [17]. Hence, when the generation of reactive free radicals overwhelms the antioxidant defense, lipid peroxidation of the cell membrane occurs. This causes disturbances in cell integrity leading to cell damage/death.

CONCLUSION

Longterm exposure to organophosphorous pesticides causes oxidative stress resulting chronic diseases in sprayer population.

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