VITAMIN E SUPPLEMENTATION ON BIOCHEMICAL CHANGES OBSERVED IN AGRICULTURAL FIELD WORKERS EXPOSED TO DIFFERENT CLASSES OF PESTICIDES

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ABSTRACT

A total of 41 healthy male pesticide sprayers exposed to different classes of pesticides for 3-5 years were compared with 21 controls matched for age and economic status with respect to free radical generation, lipid peroxidation, antioxidant status, cholesterol, lipoprotein status and haematological profile. Plasma lipid peroxidation was estimated in the form of thiobarbituric acid reactive substances (TBARS) produced. Significant increase in TBARS was observed in sprayers population when compared with control subjects and the level of TBARS increased with increase in the duration of exposure. The levels of antioxidants such as glutathione (GSH) were significantly depleted, whereas those of superoxide dismutase (SOD) were remarkably increased than control population. Significant reduction in total cholesterol, alteration in lipoprotein fractions and nonsignificant changes in hematological parameters were observed. These results suggested that exposure to pesticidal residual drift augments the free radical generation, and lipid peroxidation. Decline in non-enzymatic antioxidant and elevation of enzymatic antioxidant were observed. Supplementation of α -tocopherol for 45 days resulted in the partial restoration of these biochemical changes produced by pesticides.

KEY WORDS : TBARS, Vitamin E, Pesticide, Cholesterol, Haematology.

INTRODUCTION

Pesticides are continuously dumped in the agricultural field in order to enhance food production by eradicating some unwanted pest or insect vectors. Our present study correlates with three different classes of pesticides namely organophosphates (OP), organochlorine and carbamates. These pesticides differ in chemical nature, physico-chemical properties, mode of entry, uptake by body and metabolism (1).

Several studies have shown an association between health effects and exposure to insecticides which include disorders of cardiovascular system, nervous system, reduced lung function. Disorders like dermatitis, headache and nausea have also been reported. Abnormal electroencephalograms (EEG) were observed in some studies of farm workers

exposed to organochlorine, organophosphate and carbamate pesticides. Altered liver enzyme activities have been reported among pesticide workers exposed to organophosphorus pesticides alone or in combination with organochlorine or other pesticides (2).

More recently, various studies from several parts of the world revealed the toxic effects of pesticides on human beings especially by elucidating free radical mechanism, which can be confirmed by the direct measurement of lipid peroxidation byproducts such as malondialdehyde (MDA), lipofucins, diene conjugates and oxido-redox parameters such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and reduced glutathione (GSH) (3).

α -Tocopherol, a proven chain-breaking antioxidant has been used in medicine in the treatment of cataract, atherosclerosis, cancer, tardive dyskinesia and peripheral neuropathy, but no one carried out

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on its effect on sprayers elsewhere. Few reports assessing immunologic and hematologic changes in pesticide sprayers who are occupationally exposed to individual pesticides are available (4) but no detailed report is available on lipid parameters and antioxidant status of farmers occupationally exposed to many different classes of pesticides simultaneously and the role of α -tocopherol supplementation to these sprayers.

MATERIALS AND METHODS

Subjects

Sixty two male subjects were taken up for the present study. Among them, forty one occupational male sprayers with the age group of 22-34 years have been randomly selected based on oral questionnaires from rural area of Chidambaram Taluk, Cuddalore district, Tamil Nadu, who have been in this field for 3 to 5 years with two minimum exposures in a fortnight. The control group consisted of twenty one male healthy volunteers from department of Biochemistry, Annamalai University, Annamalai nagar, who were not performing spraying activity and did not have any kind of pesticide exposure. Consent was obtained from both sprayers and control group persons.

Blood was collected after an overnight fast in heparinised and unheparinised tubes in the sprayfield. Plasma, serum and hemolysate were prepared and stored at -4°C for further analysis as per the details given below.

Methods

For lipid peroxidation products, thiobarbituric acid was added to plasma sample under acidic condition, and the absorption of colour that developed after heating was estimated spectrophotometrically at 535 nm (5). 1, 1' 3, 3'-tetramethoxy propane was used as an internal standard and the concentration was expressed in nmol of malondialdehyde (MDA) per ml of plasma.

Plasma reduced glutathione was estimated according to the method of Beutler and Kelley (6). This method was based on the development of yellow

colour when 5,5'-dithio-bis 2-nitrobenzoic acid (DTNB) was added to compounds containing sulphhydryl groups.

Superoxide dismutase (SOD) activity was assayed according to the method of Kakkar et al (7) using phenazine methosulphate and nitro blue tetrazolium for colour development.

The total serum cholesterol was estimated by the method of Zak (8) and the HDLc was analysed in an Autoanalyser using the reagent kit obtained from Boehringer Mannheim, Germany. LDLc was calculated using the formula (9) $\text{LDL}_c = \text{Total cholesterol} - (\text{HDL}_c + \text{TG}/5)$. Serum total lipid content was calculated as recommended by Phillips and colleagues (10). $\text{Total serum lipid (mg/dl)} = 2.27 \times \text{serum cholesterol (mg/dl)} + \text{serum triglycerides (mg/dl)} + 0.623$. Glucose, urea, uric acid and creatinine were assayed in Autoanalyser using the reagent kit (Boehringer Mannheim, Germany).

RESULTS AND DISCUSSION

The present study demonstrated a significant but small increases in Hb ($p < 0.05$), eosinophil ($p < 0.01$) and decrease in Hct ($p < 0.01$) in association with increasing pesticide use in a cohort of Chidambaram Taluk farmers. (Tables 1 & 2). These findings are in agreement with the investigation of blood chemistry of Nebraska farmers [4] who applied pesticide to their fields. Myeloid: erythroid ratios were markedly increased in bone marrow of beagle dogs exposed daily to the OP insecticide diazinon [11] and erythroid progenitor cells were markedly reduced in the bone marrow of mice, one week after the treatment with OP insecticide, parathion (12). This change was due to a reduction of erythroid element in the bone marrow of pesticide sprayers.

In our present study GSH ($P < 0.001$) and cholesterol ($P < 0.01$) (Table 3) were significantly decreased whereas the plasma TBARS and SOD were significantly elevated ($P < 0.001$) when compared to control subjects. Glutathione is responsible for the protection of cell against oxy-free radicals (13). Decreased level of GSH could be due to the reaction

Table 1. Age, height, weight, blood pressure (BP), pulse rate, body mass index (BMI), body percent fat and fat mass of unexposed population, exposed population and antioxidant supplemented population.

	Age (years)	Height (cm)	Weight (kg)	Systolic BP (mm/Hg)	Diastolic BP (mm/Hg)	Pulse (rate/min)	BMI (Kg/m ²)	Body fat (%)	Fat mass (kg)
Un-exposed Population	23.2±1.2	167.2±3.4	59.2±4.1	81.0±2.4	121.9±2.4	71.95±1.4	21.2±1.5	61.8±4.8	13.0±1.4
Pesticide exposed population	29.5±4.2	163.0±6.3*	60.1±7.02	80.3±1.9	121.0±2.5	71.50±2.0	22.7±2.9	60.1±3.5	13.7±2.1
Antioxidant supplemented population	27.9±3.3	161.86±5.8**	60.8±7.1	80.7±1.69	121.3±2.2	71.00±1.0	23.3±3.0*	61.1±3.5	14.2±2.2

Values are statistically significant when compared to control ** $p < 0.01$ * $p < 0.05$

Table 2. Hematological profile of un-exposed population, pesticide exposed population and antioxidant supplemented population.

	Un-exposed population (n=21)	Pesticide exposed population (n = 41)	Antioxidant supplemented population (n=41)
Hb (gm%)	14.6 ± 2.1	15.6 ± 2.8*	15.7 ± 6.8
RBC count (cells/cmm)	8.4 ± 1.2	8.6 ± 2.2	8.6 ± 0.8
Polymorpho neutrophils (%)	54.7 ± 7.4	51.4 ± 9.6	50.2 ± 9.4
Leucocytes (%)	33.1 ± 8.4	32.2 ± 6.5	34.7 ± 8.1
Eosinophils (%)	12.2 ± 3.6	14.26 ± 3.4**	13.3 ± 4.3
ESR (mm/hr)	5.5 ± 1.7	6.2 ± 3.6*	5.2 ± 1.7
Hct (%)	44.6 ± 3.7	43.5 ± 2.6*	43.1 ± 1.7

Values are statistically significant when compared to control ** $p < 0.01$, * $p < 0.05$

of GSH with methyl group of pesticide to form conjugation product (GSCH₃) (14). But on α -tocopherol supplementation, the GSH was restored to near normal value. The increased ($P < 0.001$) formation of peroxide (TBARS) observed in the sprayer population could be due to the increased peroxidation of membrane (15). α -tocopherol (10 weeks) supplementation has also been shown to suppress the index of oxidative stress in smokers and non-smokers (16). Increased activity ($P < 0.001$) of SOD may be due to the production of reactive oxygen species. It is known that increased SOD activity is a defense

Table 3. Plasma TBARS, GSH, SOD and cholesterol of un-exposed population, pesticide exposed population and antioxidant supplemented population.

	Un-exposed population (n=21)	Pesticide exposed population (n = 41)	Antioxidant supplemented population (n=41)
Plasma TBARS (nmol/ml)	5.5 ± 1.9	13.6 ± 6.3***	8.6 ± 4.3**
Glutathione (mg/dl)	33.6 ± 6.7	23.14 ± 5.2***	28.7 ± 6.8*
SOD (units/mg Hb)*	8.5 ± 2.5	17.7 ± 6.4***	13.4 ± 3.3***
Cholesterol (mg/dl)	188.7 ± 16.6	146.3 ± 11.4**	164.4 ± 23.7

a = one unit of SOD is expressed as 50% inhibition of NBT under experimental condition

Values are statistically significant when compared to control

*** $p < 0.001$, ** $p < 0.01$ * $p < 0.05$

mechanism against increased oxidative stress in the process of attempted cellular repairs (16). The restoration of antioxidant enzyme on α -tocopherol supplementation is an index of improvement in the redox status as seen by the increment in the level of GSH in sprayer population.

Levels of the lipoproteins HDLc, LDLc, and VLDLc in the three groups are shown in table 4. while there was no significant change in HDLc between the three groups the levels of LDLc and VLDLc were lower in the pesticide and α -tocopherol supplemented groups. Like wise total fat was slightly less in pesticide as well as α -tocopherol

supplemented groups. Pesticides are known to cause fatty liver in rats (17). Fatty liver is the result of an imbalance between the rate of synthesis and the rate of utilisation of hepatic triglycerides. Regular farm activity plays a vital role in lowering the serum cholesterol level. It has been reported that regular physical exercise had favourable effects on serum lipids, lipoprotein level especially in reducing total

Table 4. Lipoprotein fraction of un-exposed population, pesticide exposed population and antioxidant supplemented population.

	Un-exposed population (n=21)	Pesticide exposed population (n = 41)	Antioxidant supplemented population (n=41)
Plasma TBARS	5.5 ± 1.9	13.6 ± 6.3***	8.6 ± 4.3**
HDL _c (mg/dl)	39.1 ± 5.5	39.4 ± 3.2	38.3 ± 4.6
LDL _c (mg/dl)	124.9 ± 13.62	96.4 ± 12.3*	105.7 ± 27.2***
VLDL _c (mg/dl)	39.1 ± 10.3	28.3 ± 5.1**	22.4 ± 4.7*
Total fat (mg/dl)	560.3 ± 26.3	482.2 ± 22.2***	502.6 ± 28.9***

Values are statistically significant when compared to control

****p* < 0.001, ***p* < 0.01, **p* < 0.05

cholesterol (19). Role of α -tocopherol in lipo-protein metabolism is clearly not known. However, α -tocopherol concomitantly decreases the oxidation of LDLs fraction and thereby reducing the pesticide associated effects in human being.

Table 5. Glucose, urea, uric acid and creatinine levels of un-exposed population, pesticide exposed population and antioxidant supplemented population.

	Un-exposed population (n=21)	Pesticide exposed population (n = 41)	Antioxidant supplemented population (n=41)
Glucose (mg/dl)	92.6 ± 3.6	86.6 ± 8.7**	87.3 ± 8.2*
Creatinine (mg/dl)	1.2 ± 0.2	1.1 ± 0.3 ^{NS}	1.3 ± 0.3*
Urea (mg/dl)	20.5 ± 4.5	22.7 ± 4.6 ^{NS}	21.7 ± 3.9
Uric acid (mg/dl)	5.5 ± 1.1	5.6 ± 1.0 ^{NS}	3.8 ± 0.9***

Values are statistically significant when compared to control **p* < 0.05, ***p* < 0.01, ****p* < 0.001

Among the other parameters studied (Table 5) uric acid level was less in antioxidant supplemented group when compared with the other two groups. Its implications are not known. There were no significant differences in the levels of glucose, creatinine and urea between the three groups.

In conclusion α -tocopherol seems to have reversed, at least partially, the changes brought about by pesticides on the lipid peroxidation products, antioxidants and serum lipid profile.

REFERENCES

1. World Health Organisation (WHO)/United Nations Environmental Programme (UNEP) (1990) Public Health Impact of pesticides used in Agriculture, Geneva, World Health Organisation.
2. International Agency For Research in Cancer (IARC) (1991) Occupational exposure in insecticide application and some pesticides. In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Lyon: International agency for Research on Cancer p. 53-60.
3. Stevenson, D.E., Kehrer, J.P., Kolaga, K.L., Walborg, E.F. Jr. and Klaunig, J.E. (1995) Effects of dietary antioxidants on dieldrin induced hepato-toxicity in mice. *Toxicol. Lett.* 75 (1-3), 177-183.
4. Casale, G.P., Scott, D.M., Anderson, J.R., Edward, F., Vitzthum and E. Hold, R.E. (1998) A preliminary study of Immunologic and Haematologic profiles of peripheral blood from Nebraska farmers who apply pesticides to their field. *Clinical Toxicology* 36 (3), 18-194.
5. Nichans, W.G. Jr. and Samuelson, B (1968) Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Brit. J. Biochem.* 6, 126-130.

6. Beutler, E. and Kelley, B.M. (1963) The effect of sodium nitrate on red cell glutathione. *Experientia* 19, 96-97.
7. Kakkar, P., Das, B. and Visvanathan, P.N. (1984) A modified spectrophotometric assay of superoxide dismutase. *Ind. J. Biochem. Biophys.* 21, 130-132.
8. Zak, B., Boyle, A.J. and Zlatkis, A. (1953) A method for the determination of serum cholesterol. *J. Clin. Med.* 41, 486-492.
9. Friedwald, W.T., Levy, R.J. and Fredrickson, D.S. (1972) Estimation of concentration of LDL- c in the plasma without the use of preparative ultracentrifuge. *Clinical Chemistry* 18, 449-453.
10. Philipps, D.L., Pirkle, J.L., Burse, V.W., Bemert, J.T., Henderson, O. and Needham, L.L. (1989) Chlorinated hydrocarbon levels in human serum: Effects of fasting and feeding. *Arch. Environ. Contam. Toxicol.* 18, 495-500.
11. Earl, F.L., Melveger, B.E., Reinwall, J.E., Bierbower, G.W. and Curtis, J.M. (1971) Diazinon toxicity - Comparative studies in dogs and miniature Swine. *Toxicol. Appl. Pharmacol.* 18, 285-295.
12. Gallicchio, V.S., Casale, G.P., Bortholomew, P.M. and Watts, T.D (1987). Altered Colony - forming activities of bone marrow hematopoietic stem cells in mice following short-term *in vivo* exposure to Parathion. *Int. J. Cell. Cloning* 5, 231-241.
13. Urano, s., Hoshi-Hashizume, M., Tochigi, N., Matsuo, M., Shiraki, M. and Ito, H. (1991) Vitamin E and the susceptibility of erythrocytes and reconstituted liposomes to oxidative stress in aged diabetics. *Lipids* 26, 58 -61.
14. O'Leary, K.A. and Tracy, J.W. (1991) Glutathione-S-transferase catalysed detoxification of Dichlorvos. *Exp. Parasitol.* 72, 355 - 361.
15. Calzada, C., Bruckdorfer, K.R., Rice Evans, C.A. (1997) The influence of antioxidant nutrient on platelet function in healthy volunteers. *Atherosclerosis* 128 (1), 97-105.
16. Brown, K.M., Morrice, P.C. and Duthie, G.G. (1994) Vitamin E supplementation suppress index of lipid peroxidation and platelet counts in blood of smokers and non-smokers but plasma lipo-protein concentration remain unchanged. *Am. J. Clin. Nutr.* 60 (3), 383 - 387.
17. Tarada, M., Mizuhashi, F., Tomita, T. and Murata, K. (1998) Effect of meparipyrin on lipid metabolism in rats. *J. Toxicol. Sci.* 23 (3), 235 - 241.
18. Vani, M., Reddy, G.P., Reddy, G.R., Thyagaraju, K. and Redanna, P. (1990) Glutathione-S-transferase, superoxide dismutase, xanthine oxidase, catalase, glutathione peroxidase and lipid peroxidation in the liver of exercised rats. *J. Biochem. Inter.* 21 (1), 17-26.
19. Sutherland, W. and Woodhouse, S.P (1980) Physical activity and Plasma lipoprotein lipid concentration in men. *Atherosclerosis* 37, 285-292.