EVALUATION OF PESTICIDES INDUCED TOXICITY BY OXIDATIVE STRESS AND INFLAMMATORY BIOMARKERS

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ABSTRACT

Objective: To evaluate pesticides induced toxicity by oxidative stress and inflammatory biomarkers among tobacco farmers in district Sawabi of Pakistan.

Study Design: Cross sectional descriptive study.

Place and Duration of Study: The study was conducted in district Sawabi, NWFP, Pakistan from Jan 2006 to March 2008.

Material and Methods: Total of 109 adult male consisting of 55 tobacco farmers and 54 unexposed subjects were included from district Sawabi, NWFP, Pakistan. Plasma Butyrylcholinesterase (BChE) and Gamma Glutamyltransferase (GGT) were measured on Vita Lab Selectra E. Plasma Malondialdyhyde (MDA) and Nitric oxides (NO) were estimated by formation of thiobarbituric acid and the Griess reaction respectively. Serum C-reactive protein (CRP) was assayed on Immulite-1000.

Results: The tobacco farmers age ranged from 16-72 years. Plasma BChE mean (SD) levels were significantly decreased to 5596 (929) as compared to 6821 (1365) U/L in the pesticides exposed farmers (P<0.001). Oxidative stress and inflammatory markers in the pesticides exposed farmers were significantly raised as compared to control which are given as mean (SD) (a) GGT 24(6) vs 17(4) U/L (b) Nitrate 34.27(19.71) vs 21.35 (11.57) umol/L (c) CRP 1.44 (0.860) vs 0.911(0.538) mg/L and MDA 4.71 (2.01) vs 3.27 (0.94) nmol/ng (P<0.001) respectively. The plasma BChE levels showed a significant inverse correlation with plasma MDA (r=-0.39), nitrate (r=-0.44) and CRP(r=-0.40).

Conclusion: Apart from plasma BChE inhibition, pesticide exposure enhanced oxidative stress and inflammatory markers in the tobacco farmers. Plasma BChE has inverse correlation with lipid peroxidation, nitrate production and inflammatory biomarkers which might be used for monitoring of pesticides induced risks in occupational workers.

Keywords: Pesticides, oxidative stress, BCh E, nitrate, CRP, MDA

INTRODUCTION

Pesticides are used extensively in agriculture to enhance food production by eradicating unwanted insects. The wide spread use of pesticides in agriculture has caused environmental pollution and potential health hazards [1]. Pesticides produce adverse biological effects through the active ingredients and associated impurities [2]. Exposures to pesticides produce depression in plasma BChE activity in farmers [3] which can be used for their risk assessment. The irrational use of pesticides in agriculture may affect health of farmers by producing reactive oxygen specie (ROS) leading to increased oxidative stress and inflammatory cytokines in the blood. Cellular antioxidant status also determines the susceptibility to oxidative damage due to ROS [4].

Pesticides induce oxidative stress as well as alter the defence mechanisms of detoxification and scavenging enzymes [1, 5]. These toxic compounds impair the cellular function, enzymes activity and produce cytotoxic changes through generation of ROS [6]. These free radicals also damage the cell components including proteins, lipids and DNA [7]. In humans, organophosphates (OP),

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and pyrethroids (PT) exposure have been linked to lipid peroxidation [8, 9]. Malondialdehyde (MDA) is the most frequently used biomarker of lipid peroxidation [10]. Chronic over production of ROS lead to redox imbalance leading to increased Gamma Glutamyltransferase (GGT). GGT activity is regarded as an early marker of oxidative stress in humans [11].

Exposure to pesticides may also contribute to modulate immune system leading to adverse health consequences [12]. The involvement of immune biomarkers in pesticide related health studies seems to be of considerable value. A high sensitivity C - reactive protein (hS-CRP) concentration has been used for the assessment of disease progression through a pro- inflammatory property [13, 14]. Persistently increased hS-CRP represents a sensitive and valuable marker of an ongoing disease process [15].

Pesticides specifically target constitutive nitric oxide synthase (cNOS) mediated processes [16]. Nitric oxide is also an important biomarker of inflammation. A role for nitric oxide has been postulated as a potent mediator of cellular damage in a wide range of conditions. Cytotoxicity attributed to NO is due to peroxynitrite and superoxide anion [17].

In our country tobacco farmers are over exposed to different types of pesticides during work in the field. Adverse effects of pesticides residues on hepatic and renal biochemical markers in the tobacco farmers have been recently reported [18]. Unfortunately, very little work has been done on the effects of pesticides on oxidative stress and inflammatory cytokines in this high risk group of farmers. Thus the study was planned to evaluate pesticide induced oxidative stress and inflammatory biomarkers among the exposed tobacco farmers in district Sawabi, North West Frontier Province (NWFP) of Pakistan.

MATERIALS AND METHODS

This cross-sectional descriptive study was conducted in district Sawabi, North West Frontier Province (NWFP) of Pakistan. Two tobacco growing villages Chota Lahore and Jangen Nath were randomly selected. Six different types of pesticides were used for the high yield of tobacco crop in Sawabi area. The carbamate (CM), marketed under the tradename of Lannate (Methomyl; WHO toxicity class IB highly hazardous), Larvin (Thiodicarb; Class II moderately hazardous) were the commonly reported pesticides used by 70% of the farmers. Cypermethrin, (Toxicity Class II; moderately hazardous), marketed under the same trade name was the second most commonly used pesticide. The organophosphate (OP), Methamidophos, marketed under the trade name Grip belonging to Class IB highly hazardous was also used. The organochlorinated compounds Thiodan (endosulphan) and Confidor (imidacloprid; toxicity Class II moderately hazardous) were used occasionally.

This study was approved by institutional review committee of AM College, Rawalpindi and Higher Education Commission (HEC) Pakistan.

Subjects:

Total 109 adult males consisting of 55 tobacco farmers and 54 control subjects from same area were included in the study after an informed consent. The participants were male, age range from 16 to 72 years. The pesticides exposed tobacco farmers were randomly selected from the farms on the basis of their full time active involvement in preparation, storage and spraying of the pesticide on the tobacco crop. Medical history and physical examination of the subjects were carried out before the start of study. Farmers suffering from diabetes mellitus, hypertension, chronic renal failure, viral hepatitis or other chronic illness not related to pesticide exposure were excluded from the study.

Biochemical Analysis:

Blood sample (5ml) was collected in heparianized tubes from both control and exposed subjects. Plasma was separated by

centrifugation at 1500g for 15 minutes. Biochemical analysis was carried out at the laboratory of Army Medical College, Rawalpindi.

Plasma BChE activity was measured as per Ellman's colorimetric method by using GD kits (Italy) on Selectra E auto analyzer (Vita lab, Netherlands) following the standard procedures [19]. Serum GGT activity was assessed by a kinetic colorimetric assay at 37oC and expressed as unit per litre according to IFCC procedures [20]. The coefficient of variance (C.V) of the methods was found to be 3-5%.

The extent of lipid per oxidation in whole blood was assayed by measuring the formation of thiobarbituric acid reactive substance by using the method describes by Gavino [21]. Serum MDA was measured by heating samples with thiobarbituric acid at low pH. Quantitative absorbance of pink color was measured at 532 nm on photometer (Schematize, Japan).

Nitrate was measured by using Griess reagent by using the colorimetric assay kit (Cayman, UK) at 540nm on ELISA reader [22]. Plasma samples were diluted fourfold with distilled water and deproteinized by adding 1/20th volume of zinc sulfate (300 g/L) to give final concentration of 15 g/L. After centrifugation at 10,000 g for 5 min at room temperature (or 1000 g for 15 min) 100 uL of supernate was applied to a micro titer plate well followed by 100 uL of a Griess reagent (1g/L sulfanilamide), 25 g/L phosphosic acid, and 0.1 g/L naphthlethylenediamine. After 10 min of color development at room temperature, the absorbance was measured on a micro plate reader (Titertek Multishan MCC 1340; Flow Lab MC Lean VA) at a wavelength of 540 nm. Each sample was assayed in duplicate wells.

CRP was analyzed on Immulite 1000 by using DPC kit (USA) [23]. C.V was 4.8%.

Statistical Analysis:

The data was analyzed by using standard SPSS software version-15 (SPSS Inc, Chicago). BChE, oxidative stress and inflammatory biochemical markers were expressed as mean and (SD). Comparison of mean biochemical changes in pesticides exposed farmers and control subjects were analyzed with independent samples t-test. Spearman's coefficient correlation was calculated between plasma BChE and oxidative stress markers including serum GGT, MDA, nitrate and CRP. P-value of <0.05 was considered significant.

RESULTS

The tobacco farmers age ranged from 15 to 72 years with mean age of 29 years. Mean (SD) plasma BChE level in the pesticide exposed tobacco farmers was significantly reduced 5596 (929) U/L as compared to 6821 (1365) U/L in healthy control ($p<0.001$). The box plot of the BChE result (figure). Out of 55 tobacco farmers, 17(31%) had plasma BChE levels below the reference range (5172-10430 U/L).

The pesticide exposed tobacco farmers had increased oxidative stress indicated by significantly raised serum MDA and GGT levels as compared to healthy volunteers (table-1). Most of the farmers had GGT within the reference range of 11-61 U/L. Twenty (36%) tobacco farmer had MDA above the reference range of 1.8-4.7 mmol/mL. Serum nitrate and CRP levels were also increased due to nonspecific inflammatory changes in the exposed farmers as compared with controls ($p<0.001$). Nine (16%) tobacco farmer had nitrate above the reference range 4.0-45.3 whereas 33(37%) tobacco farmers had CRP above the reference range of <1 mg/L. Our study had revealed significant inverse correlation between BChE with GGT, MDA, nitrate and CRP due to oxidative stress and inflammation in the pesticides exposed tobacco farmers (table-2).

DISCUSSION

The health effects of pesticides exposure are difficult to monitor in the farmers especially when mixture of pesticides are used over a period of time. Our tobacco farmers had significantly low BChE activity indicating significant OP and CM exposure.

Table-1: Oxidative stress and inflammatory biochemical markers in pesticides exposed tobacco farmers as compared to controls (n=109).

Biomarkers	Exposed (n=55) Mean (SD)	Unexposed (n=54) Mean (SD)	P-value
MDA (umol/L)	4.71 (2.0)	3.27 (0.94)	0.001
GGT(U/L)	24 (6)	17 (4)	0.001
Nitrate (nmol/L)	34 (20)	21 (12)	0.001
CRP (mg/L)	1.44 (0.86)	0.911 (0.54)	0.001

Table-2: Correlation between BChE with serum GGT, MDA, nitrate and CRP in pesticides exposed tobacco farmers (n =55).

Parameters	r	P
GGT	-0.28	0.004
MDA	-0.39	0.003
Nitrate	-0.44	0.001
C-Reactive Protein	-0.40	0.003

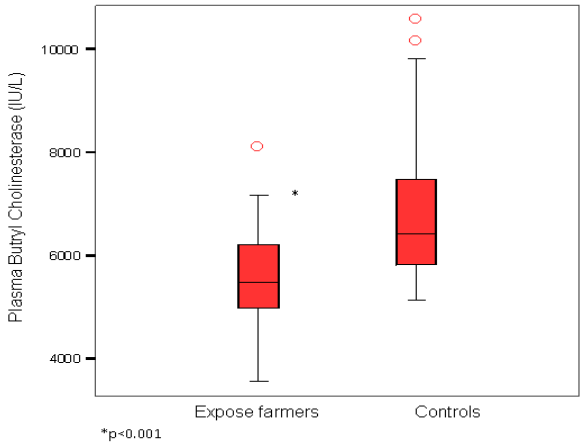

Figure: Plasma Butryl cholinesterase in pesticides exposed tobacco farmers as compared to controls (n=109).

Low levels of BChE have been demonstrated by many researchers in prolong pesticide exposure [24, 25]. Rambabu (2007) also reported a progressive fall in plasma AChE levels in exposed farmers compared to unexposed individuals [26]. Thus depression in plasma BChE activity has revealed significant pesticide induced toxicity in our tobacco farm workers. Currently AChE is the only biomarker used for monitoring the agricultural workers, applicators and handlers.

Toxic effects of pesticide on human beings especially by omit radical production can be confirmed by the direct measurement of lipid per oxidation by-product Malondialdyhyde (MDA) [27]. Our study revealed a significant rise in MDA levels in exposed farmers than in controls. The results of our study were consistent with other studies suggesting that pesticides increase oxidative stress in humans [8, 28]. There is increasing evidence that OP and CM induced oxidative stress through the generation of free oxygen radicals, leading to lipid peroxidation and DNA damage [1, 29-31]. Muniz et al., (2007) reported MDA levels 4.9 times and 24 times higher in farm workers and applicators respectively than in controls [9]. Pesticides induce a wide array of human health effects through oxidative stress causing cytogenetic damage and carcinogenicity [32].

In our study GGT levels were also found to be significantly raised in the pesticide exposed farmers as compare to controls. GGT is an enzyme mainly present in cell membranes and is susceptible to damage due to the presence of pesticides [33]. Our results consistent with the studies in which human blood samples obtained from pesticide exposed individual showed raised GGT levels [34-35]. Altuntas also reported raised GGT in rats exposed to methidathion [36]. Although the liver enzymes are not completely specific, an increase in their activities reflects active liver damage. It is also seen that there is a positive correlation of GGT with the oxidative stress markers [11].

We have also found in our study that serum nitrate levels were significantly high in exposed farmers than controls; Occupational exposure to trichloroethylene (TCE) cause increase in the production of NO responsible for TCE-induced erythema and skin inflammation in human [37]. Pesticides Methoxychlor (MXC) stimulate the production of NO and proinflammatory

cytokines and can up-regulate the gene expression levels via NF-KB transactivation and thus possess an inflammatory potential. [38]. Dur Zong also reported raised nitric oxide levels among the rats that have been exposed to pesticides in a dose dependant manner [39]. Carbontetrachloride also induce oxidative stress and increases inducible NOS protein expression along with other inflammatory mediators among adult rats [40].

Our study also revealed raised CRP in exposed farmers as compare to controls. C-reactive protein is a marker of inflammation and is linked to various pathological processes associated with chronic inflammation. Following an acute phase stimulus, CRP values may increase by as much as 10,000 fold by hepatic synthesis regulated by pro inflammatory cytokines, especially interleukin-6 (IL-6) [13]. Inflammation occurs as a part of the body defense against infections or injury. The body triggers the inflammatory cytokines such as interleukin 6, which then set off the production of CRP by the liver. CRP exhibited three to five fold elevations following intoxication suggesting that they may be sensitive and highly effective biomarker of pesticide exposure [41]. CRP can be use for screening; monitoring and detecting inter current infections [13]. Pesticides could induce variety of cancers through an immunological mechanism [42]. These results indicate that there are other mechanisms of cell disruption in associations with pesticide exposure and chronic diseases.

Our study revealed an inverse correlation between BChE inhibition and lipid per oxidation. A reduction in activity of BChE is correlated well with lipid peroxidation following subchronic and chronic OPs exposure [28, 43]. Another study in India revealed significantly higher levels of MDA with inhibited AChE activity among OPs poisoned patients [29]. Singh also reported similar correlation among pesticides sprayers in mango orchards [8].

The alteration in biochemical profile due to pesticide exposure supports our previous clinical findings. This study suggests oxidative stress and inflammatory biomonitoring of pesticide exposed farmers should be done at regular intervals to detect early adverse changes. Further studies are needed to establish the role of pesticides induced carcinogenicity among our tobacco farmers.

CONCLUSION

Pesticide exposure is accompanied by depression of BChE, increased concentration of biochemical oxidative stress and inflammatory markers in the tobacco farmers. Plasma BChE has inverse correlation with lipid peroxidation, nitrate production and inflammatory biomarkers which might be used for monitoring of pesticide induced risks in occupational workers.

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