Original Article

Oxidative stress indices in Nigerian pesticide applicators and farmers occupationally exposed to organophosphate pesticides

Yaqub A Surajudeen, Rahamon K Sheu, Kosoko M Ayokulehin[1], Arinola G Olatunbosun

Department of Chemical Pathology, [1]Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria

Abstract

Background: Reports have clearly indicated the role of oxidative stress in the pathogenesis of organophosphate pesticides (Op) toxicity. However, there is dearth of information on which group of the farm workers is more at risk of Op-induced oxidative stress. **Aim:** This study determined serum levels of malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), myeloperoxidase (MPO), nitric oxide (NO), and serum activity of acetylcholinesterase (AChE) in farm workers exposed to Op. **Subjects and Methods:** A total of 60 (30 pesticide applicators and 30 farmers) and 30 apparently healthy non-farmers who were nonexposed to Op (controls) were recruited into this study. Serum activity of AChE was determined using high performance liquid chromatography (HPLC), while serum levels of MDA, GSH, and NO and serum activities of CAT, MPO, GPx, and superoxide dismutase (SOD) were determined colorimetrically. **Results:** Serum activities of AChE and CAT were significantly lower, whereas MPO activity was significantly higher in pesticide applicators compared with controls. Similarly, farmers had significantly reduced serum AChE activity and significantly raised MPO activity compared with controls. However, serum activities of AChE, CAT, and MPO were significantly lower, whereas mean level of MDA was significantly higher in pesticide applicators compared with farmers. **Conclusion:** This study shows that Op applicators are more exposed to oxidative stress than farmers, thus Op applicators require increased antioxidant supplements than farmers.

Key words: Farm workers, oxidative stress, pesticide applicators

Submission: 03-10-2013 **Accepted:** 19-03-2014

Introduction

Different types of pesticides have been used in agricultural practice in order to enhance food production. These pesticides differ greatly in their modes of action, uptake by the body, metabolism, elimination from the body, and toxicity to humans.[1] Among pesticides, organophosphate pesticides (Op) have been widely used, as these compounds are nonpersistent in the environment.[2] Although Op are frequently used to spray cocoa, cashew, and mango plantations in south-west Nigeria, their associated poisoning is still a public health challenge even in the entire Africa.[3]

Occupational exposure to Op is usually through skin absorption and inhalation as many Op applicators do not use face masks and other protective devices.[4] Most Op pesticides exert toxicity on the target and nontarget organs through inhibition of the activity of acetylcholinesterase (AChE) in the nerve and muscle tissues.[5] Hence, determination of serum activity of AChE activity has been the standard marker of exposure to Op.[6] Organophosphate binds with cholinesterase enzyme and inhibits the activity of the enzyme by irreversible phosphorylation. This results in elevated levels of acetylcholine thus stimulating the muscarinic and nicotinic receptors resulting in consequent toxicity.[7,8]

Address for correspondence: Prof. Arinola G Olatunbosun, Department of Chemical Pathology, Immunology Unit, University of Ibadan, Ibadan, Nigeria.
E-mail: drarinolaog64@yahoo.com

Access this article online	
Quick Response Code:	Website: www.ijabmr.org
	DOI: 10.4103/2229-516X.140730

Reports on the adverse health impacts of exposure to OP have been attributed to inhibition of cholinesterase activity. However, recent findings have demonstrated that the inhibition of cholinesterase itself cannot account for the wide range of disorders that have been reported following Op exposure.[9] Hence, oxidative stress has been implicated as one of the mechanisms for the adverse health effects of Op exposure. Op has been reported to induce oxidative stress through increased levels of reactive oxygen species (ROS), hydrogen peroxide (H_2O_2), nitrate (NO_3^-), and nitrite (NO_2^-).[10] Accumulation of ROS in all the region of the brain and other tissues may disturb the normal physiological function thus aggravating the toxicity symptoms of Op.

Toxicity of Op has been reported to have adverse effects on the hematological and biochemical systems of human body.[11] Similarly, Owoeye *et al*.,[12] reported that animals exposed to Op had histologically proven damaged kidneys and lungs. Several mechanisms have been implicated in Op toxicity; however, induction of ROS is considered as an important mechanism.[13] Earlier reports showed that malondialdehyde (MDA) level was significantly elevated in carbamate and organochlorine pesticides sprayers compared with controls.[14] Similarly, Rastogi *et al*.,[13] reported reduced AChE activity and increased MDA level in organophosphorous pesticide sprayers compared with controls. These reports clearly indicate that oxidative stress plays an important role in the pathogenesis of Op toxicity.

It must be noted, however, that many of the early reports did not consider various groups of farm workers exposed to Op with a view to identifying the group that is more at risk of Op-induced oxidative stress. This study, therefore, determined the serum levels of oxidative stress indices in Op applicators and farmers exposed to Op in south-west Nigeria. This is to provide necessary information on possible need for individualized health intervention such as antioxidant supplementation and encouragement on the use of personal protective equipment (PPE) to reduce exposure.

Subjects and Methods

After obtaining informed consent from each participant and an approval (UI/EC/11/0107) from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Committee, a short structured questionnaire was administered on a group of farmers to determine duration and frequency of Op exposure and to obtain health state-related information. After thorough screening, 60 farm workers exposed to Op for at least three times in a week for not less than 10 years (30 Op applicators and 30 farmers) (47 ± 17 years) were recruited into this study. A total of 30 non-farmers (46 ± 10 years) who were apparently healthy civil servants and who had no exposure to Op served as control. Subjects with any respiratory disorder were excluded from the study. Serum activity of AChE was assayed using high performance liquid chromatography (HPLC). Serum level of catalase (CAT) was determined colorimetrically at 570 nm by measuring chromic acetate produced from the reaction of dichromate in acetic acid and H_2O_2.[15] MDA was measured by thiobarbituric acid reactive substances (TBARS) reaction in acidic medium, which on heating formed a pink complex that absorbed maximally at 532 nm.[16] Myeloperoxidase (MPO) activity was determined using the method of Bergmeyer.[17] Reduced glutathione (GSH) was estimated based on the development of a relatively stable yellow color measurable at 412 nm when Ellman's reagent was added to sulfhydryl compounds.[18] Serum SOD activity was determined by colorimetric method based on the ability of superoxide dismutase (SOD) to inhibit the autooxidation of epinephrine at pH of 10.2.[19] Nitric oxide (NO) generated from the reaction of N-1-napthylethylenediamine dihydrochloride (NED) and sulfanilamide (Griess reagent) was measured colorimetrically at 520 nm.[20]

Results

Table 1 shows the mean serum activity of AChE, duration of exposure to Op, and oxidative stress indices in pesticide applicator and farmers exposed to Op pesticides compared with controls. The mean serum activities of AChE and CAT were significantly lower, whereas MPO activity was significantly higher in pesticide applicators compared with the control subjects. Also, AChE activity was significantly lower, whereas MPO activity was significantly higher in farmers compared with the control subjects. However, serum activity of AChE, CAT, and MPO were significantly lower, whereas the mean serum

Table 1: Mean duration of exposure, levels of AChE, oxidative stress indices, and NO in applicators and farmers exposed to Op pesticides compared with controls

	Controls (*n*=30)	Applicators (*n*=30)	Farmers (*n*=30)
Mean duration of exposure (years)		13.4±1.8	12.0±1.7
AChE (IU/ml)	9.38±0.82	6.63±0.90*#	7.88±0.63*
SOD (U/mg protein)	36.62±5.09	32.26±4.90	35.34±4.79
MDA (U/mg protein)	0.458±0.041	0.489±0.028#	0.393±0.034
GSH (µg/ml)	18.33±7.11	17.17±5.83	16.50±5.11
GPx (mg/mg protein)	95.00±23.95	90.30±26.49	91.37±39.57
CAT (U/mg protein)	0.417±0.038	0.254±0.021*#	0.355±0.032
MPO (U/ml)	7.73±5.64	10.52±7.40*#	14.34±11.05*
NO (µmole)	92.77±16.59	92.40±14.39	92.33±14.98

*Significantly different from controls, #Significantly different from farmers, AChE: Acetylcholinesterase; MDA: Malondialdehyde; GSH: Reduced glutathione; GPx: Glutathione peroxidase; CAT: Catalase; MPO: Myeloperoxidase; NO: Nitric oxide; SOD: Superoxide dismutase

MDA level was significantly higher in pesticide applicators compared with farmers.

In Table 2, AChE activity had significant inverse correlation with duration of exposure and MDA in Op applicators. However, AChE activity had positive correlation with serum NO in farmers [Table 3].

Discussion

The toxic effect of Op exposure on both animals and humans is well established.[21-24] According to the World Health Organization, 3 million cases of pesticide poisoning occur every year, resulting in more than 250,000 deaths.[25] Despite this alarming figure, efforts to reduce Op-associated poisoning as well as its attendant diseases have not been successful globally.[26]

This present study shows a significant decrease in activity of AChE in pesticide applicators and farmers compared with controls as well as pesticide applicators compared with farmers. Our observation supports the earlier report of Vidyasagar *et al.*,[27] who also reported low AChE activity in the Op-exposed workers. This observation further shows that reduction in AChE activity is a common feature in Op-exposed individuals. It must be noted, however, that our observed significant reduction in AChE activity in applicators compared with farmers indicates that Op applicators are more exposed to Op poisoning than farmers.

Table 2: Correlation of AChE with duration of exposure, oxidative stress indices, and NO in Op applicator

Indices	*r* value	*P*
Duration of exposure (years)	−0.54	0.002*
SOD (U/mg protein)	0.15	0.427
MDA (U/mg protein)	−0.39	0.033*
GSH (µg/ml)	−0.05	0.795
GPx (mg/mg protein)	0.20	0.283
CAT (U/mg protein)	−0.09	0.641
MPO (U/ml)	0.26	0.173
NO (µmole)	0.01	0.984

AChE: Acetylcholinesterase; MDA: Malondialdehyde; GSH: Reduced glutathione; GPx: Glutathione peroxidase; CAT: Catalase; MPO: Myeloperoxidase; NO: Nitric oxide; SOD: Superoxide dismutase

Table 3: Correlation of AChE with duration of exposure, oxidative stress indices, and NO in farmers exposed to Op pesticides

Indices	*r* value	*P*
Duration of exposure (years)	−0.12	0.547
SOD (U/mg protein)	−0.23	0.216
MDA (U/mg protein)	0.17	0.371
GSH (µ/ml)	0.08	0.688
GPx (mg/mg protein)	−0.23	0.221
CAT (U/mg protein)	0.18	0.353
MPO (U/ml)	0.07	0.730
NO (µmole)	0.42	0.020*

AChE: Acetylcholinesterase; MDA: Malondialdehyde; GSH: Reduced glutathione; GPx: Glutathione peroxidase; CAT: Catalase; MPO: Myeloperoxidase; NO: Nitric oxide; SOD: Superoxide dismutase

Damage induced by oxidative stress primarily occurs through production of ROS, which involves 'stealing' electrons from nucleic acids, lipids, and proteins, leading to the damage of cells and consequently, disease phenomena.[28-30] In this study, we observed a significantly lower activity of CAT in Op applicators compared with the control subjects and in Op applicators compared with farmers. Similar observations were reported by Aly *et al.*,[28] and Mansour and Mossa[31] in Op-exposed farm workers. Significant reduction in CAT activity might be an indication of accumulation of H_2O_2 that requires CAT to break it down to oxygen and water. Our observation possibly suggests heightened generation of free radicals that could lead to oxidative damage[32,33] as a result of overwhelming antioxidant enzymes activities such as CAT.

MPO is a peroxidase enzyme abundantly expressed in neutrophil granulocytes.[34] It produces hypochlorous acid (HOCl) from H_2O_2 and chloride anion (Cl^-) during the neutrophil respiratory burst.[35] These products of the MPO-H_2O_2 system are powerful oxidants that can have profound biological effects.[36] We observed that serum MPO level was significantly higher in Op applicators and Op-exposed farmers compared with controls. Significantly higher MPO activity observed could be induced by the increased production of free radicals, as MPO utilizes free radicals in its bactericidal activities. However, MPO activity was significantly lower in Op applicators compared with farmers. This observation has not been reported before but it could indicate generalized impairment of enzymes involved in oxidative stress as there were significantly lower activities of CAT and AChE in this group as discussed earlier.

The observed higher level of MDA in Op applicators compared with farmers might suggest increased lipid peroxidation in Op applicators. This observation is further buttressed by the inverse correlation between AChE activity and MDA in Op applicators. This suggests that the more depressed serum AChE activity in Op-exposed farm workers, the higher the MDA level and the higher the risk of oxidative damage due to oxidative stress.[10,37] Our observation is consistent with previous studies where reduction in activity of AChE correlated with lipid peroxidation following subchronic and chronic Op exposure.[27,32] Singh *et al.*,[33] also reported similar correlation among pesticide sprayers.

There was significant negative correlation between AChE and duration of exposure in Op applicators. The inverse correlation indicates that the longer the duration of exposure to Op the lower the AChE activity and the more the risk of adverse effect of Op.

It could be concluded from this study that there is increased MDA production and reduced activities of AChE, CAT, and MPO in pesticide applicators compared with farmers. This observation is reflective of increased oxidative stress in pesticide applicators; hence, they should be encouraged to use PPE to reduce their exposure to organophosphate and increase their antioxidants supplement intake.

Acknowledgement

The authors appreciate all the participants in this study.

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How to cite this article: Surajudeen YA, Sheu RK, Ayokulehin KM, Olatunbosun AG. Oxidative stress indices in Nigerian pesticide applicators and farmers occupationally exposed to organophosphate pesticides. Int J App Basic Med Res 2014;4:37-40.

Source of Support: Nil. **Conflict of Interest:** None declared.