IJRCE

ISSN 2248-9649

International Journal of

Research in Chemistry and Environment

Available online at: www.ijrce.org

Research Paper

Estimation of Urinary Biomarker 8- OHDG in OP Pesticide Sprayers of Krishna and Guntur Districts

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(Received 30th January 2017, Accepted 20th March 2017)

Abstract: *Organophosphate pesticides can generate reactive oxygen species on DNA (both in the nucleus and mitochondria) causes a number of oxidative DNA lesions, results an oxidized form of guanosine, 8-OHdG. Exposure to pesticides both environmentally and occupationally causes a range of human health problems. It is estimated, worldwide that nearly 10,000 deaths result annually the use of chemical pesticides, with about three-fourths occurring in developing countries The main objective of the study was to evaluate the biochemical levels of 8-OHdG in spot urinary samples under the exposed OP pesticide sprayers and farm workers.*

Keywords: DNA, exposure, Organophosphate pesticides, oxidative stress, pesticide sprayers, 8-OHdG.

Introduction

India, the agricultural being the prime profession happens to be one of the major industries and require high yielding varieties of seeds for growing population Chemical pesticide poisoning in the environment is a real threat to not only human lives but also other living organisms. Exposure to pesticides both occupationally and environmentally causes a range of human health problems. Overwhelming evidence is that some of these OP Pesticides do pose potential risk to humans and other life forms and unwanted side effects to the environment more in aquatic environment[1]. In India, the first report of poisoning due to pesticides was from Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with parathion[2]. Oxidative damage is thought to be an important mechanism of damage for organophosphate pesticides[3,4]. Organophosphate pesticides can generate reactive oxygen species and alter cellular antioxidant systems[5,6,7]. Levels of products of oxidative damage in urine reflect overall damage to all tissues and organs in the body. Several DNA base oxidation products are known to be mutagenic and most abundant is the C-8 hydroxylation of the guanine base and glycol. 8-OHdG can cross the cell membrane unlike any other species that contains oxidized guanine, thus, it is usually detected in the urine or serum of patients who have diseases associated with oxidative stress[8].

Material and Methods

A questionnaire was prepared to be filled by the farmer after an informal interview to obtain data about the knowledge of utilization and handling of pesticides in an agricultural community and to estimate the levels of 8OHdG in urinary samples in the exposed OP pesticide sprayers. The target group was agricultural farmers and pesticide applicators in the agricultural areas of Krishna and Guntur District. They were initially elicited to express their own views and spontaneous responses were recorded and documented. Early morning urine samples were collected.

The agricultural workers in the present study are in the age group between 25–45 years having various exposure periods which may range between minimum 1 to a maximum of more than 10 years. According to the duration of exposure to organophosphorus pesticides, the agricultural workers i.e., the study group is divided into two groups on the basis of their

exposure as Group I the workers having acute exposure (<1 year) and Group II will include having prolonged chronic exposure (>10 years). The study group is also divided into three groups according to the type of organophosphorus pesticide (OP) sprayed. The pesticide applicators spraying only methylated OP pesticides are Group I, spraying only ethylated OP pesticides, Group II and both methylated and ethylated OP pesticide applicators spraying as Group III.

The oxidative DNA damage is quantified measuring the 8-OHdG by ELISA method. The unknown 8-OHdG samples are added to an 8-OHdG/BSA conjugate pre-absorbed on the micro plate, followed with the incubation. Then an anti 8-OHdG monoclonal antibody is added, followed by an HRP conjugate secondary antibody. Results are read at 450nm on a microplate reader. The 8-OHdG content in unknown sample is determined by comparison with predetermined 8-OHdG standard curve.

Results and Discussion

The mean values of 8-OHdG measured in the controls and exposed group are shown in table 1 and as figure: 1. There was a significant increase in the urinary level of 8-OHdG in the exposed workers in contrast to the mean value measured in the controls (17.8±3.7 vs. 10.6±4.2 µg/gm; p<0.001). The increased urinary levels of 8-OHdG were contributed by both non-smokers as well as smokers in the exposed group. Smokers both in the control as well as in the exposed group showed significantly higher values in comparison to the values obtained in non-smokers. Our results, therefore, clearly show the influence of smoking in the urinary levels of 8-OHdG. The affect of age and alcoholism on urinary levels of 8-OHdG were depicted in table 2 and as figure: 2, these findings revealed no significant increase or decrease differences in the mean values of 8-OHdG in urine samples of the controls and exposed workers.

Table 1: The levels of 8-OHdG level in controls and exposed groups

Study Group	Urinary 8-OHdG level (µg/g creatinine)	
	Mean ±SD	Range
Controls		
Non-Smokers (n=25)	9.4 ± 5.6	7.1 - 12.5
Smokers (n=25)	12.7 ± 4.8	9.5 – 15.7
Sub Total	10.6 ±4.2	6.9 – 16.1
Exposed		
Non-Smokers (n=62)	13.2 ± 4.0	10.7 – 19.5
Smokers (n=63)	20.9 ±3.8	13.5 – 23.5
Sub Total	17.8 ± 3.7	10.7 – 23.5

When the results were compared between with < 30 years and > 30 years of age groups. Similarly, the affect of alcoholism on urinary 8-OHdG levels was not discernible in this study. The affect of the period of exposure on the urinary levels of 8-OHdG are shown in table 3 and as Figure: 3.

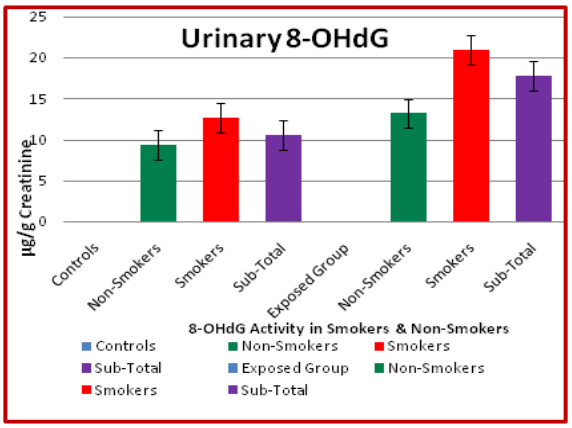

Figure 1: 8-hydroxyguanosine levels in controls and OPP Exposed Groups

Table 2: 8-OHdG in relation to age and alcohol consumption in controls & exposed group

Variables	Urinary 8-OHdG level (µg/g creatinine)	
	Control Group (n=50)	Exposed Group(n=125)
< 30 years	11.3 ± 5.8	15.7 ± 3.8
> 30 years	10.7 ± 3.2	17.1 ± 4.0
Non - Alcoholics	10.8 ± 4.2	14.8 ± 3.01
Alcoholics	11.6 ± 5.1	16.4 ± 4.8

The values are mean ± SD

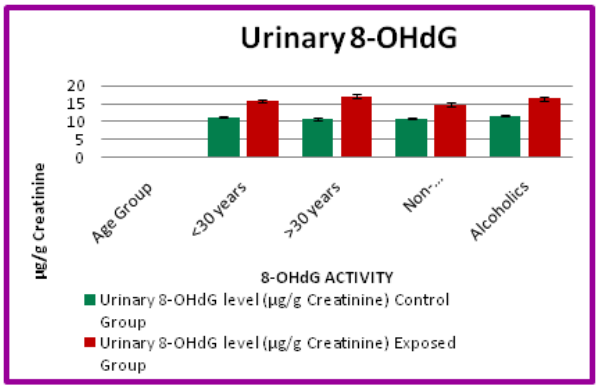

Figure 2: 8-OHdG in relation to age and alcohol consumption in Controls and Exposed Groups

The pesticide workers having more than 10 years of exposure to OP pesticides showed significantly higher values of 8-OHdG in contrast to values measured in less than 10 year exposure group. The urinary levels of

8-OHdG in different groups of pesticide sprayers in table 4 and as Figure 4.

Table 3: 8-OHdG levels in various exposure groups

Exposure Groups (n=125)	Urinary 8-OHdG level (µg/g creatinine)
< 1 year	10.8 ± 3.6
1 - 5 years	13.6 ± 4.1
6-10 years	17.7 ± 3.8
11-15 years	16.8± 4.1
16-20 years	16.4 ± 5.1

Values are mean ± SD

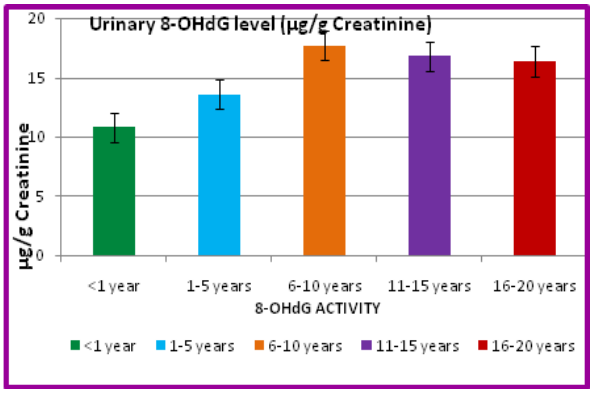

Figure 3: 8-OHdG levels in various Exposure Groups

Table 4: Urinary 8-OHdG level in pesticide workers spraying different OP's

Type of OP pesticide sprayed	Urinary 8-OHdG Level (µg/g creatinine)
Group – I (n=39)	10.6 ± 4.2
Group – II (n=41)	13.9 ± 4.8
Group – III (n=45)	20.7 ± 3.9

Values are mean ± SD

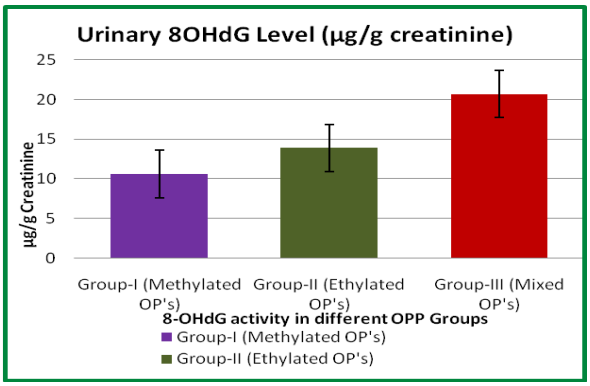

Figure 4: Urinary 8-OHdG level in pesticide workers spraying different OP's

The exposed workers spraying the mixtures of methylated and ethylated OP pesticides in mixed form showed significantly increased urinary levels of 8-OHdG (20.7±3.9) in comparison to group-I spraying only methylated OP insecticides (15.6±5.3) or ethylated OP insecticides, group – II (13.9±4.8). This suggests the additive or synergistic action of OP insecticides when mixed in variable proportions and concentration for spraying purposes.

Urinary 8-OHdG indicates the extent of Oxidative damage and is linked to many degenerative diseases. In the present study the urinary 8OHdG are given in the table 1, 2, 3, 4 and as figures 1, 2, 3, 4 a significant increase in the urinary level of 8-OHdG in the OP pesticide exposed group when compared to the controls. The increased levels of 8-OHdG were observed in both the smoker and non-smokers in the exposed groups. But smokers both in the control as well as in the exposed group showed higher values in comparison to nonsmokers. Furthermore pesticide workers having more than 10 years of exposure showed a significant increase of 8-OHdG. Exposed workers spraying the mixtures of pesticides showed more increased urinary levels of 8-OHdG. It is one of the most abundant oxidative DNA adduct[9]. It has been estimated that 8-OHdG represents about 5% of all oxidative adducts[10].

The prevalence and relative ease of measurement of 8-OHdG has made it the most popular oxidative adduct to be used in molecular epidemiology studies of increased oxidative DNA damage from occupational exposures[11-14]. In addition to being abundant, 8-OHdG is highly mutagenic resulting in predominantly G-Transversions[15,16]. 8-OHdG can be measured in hydrolyzed DNA from lymphocytes where it presents the steady state between damage and repair. Recent studies[17-21] indicated that the urinary levels of 8-OHdG can vary over a 24 hour period were correlated with our present study. Several factors could be responsible for the overall increase in urinary 8-OHdG levels observed in this study; (i) Most of the pesticide sprayers in the present study mixed and sprayed OP pesticides, re-entered fields and also lived in the farm thereby got continuously exposed to these agrochemicals. (ii) The average duration of exposure to OP pesticides in these workers was longer (12.5+/-6.20 years) then in the previously reported studies; (iii) Increased use of OP pesticides; (iv) The variations in environmental temperatures and humidity may also be responsible for enhancing the net absorption and eventual exposure to pesticides through dermal and nasal routes; (v) The most of the participants of this study did not use or wear protective gear (PPE) while mixing and spraying OP pesticides or while working in the fields and (vi) Finally these workers not only occupationally and environmentally exposed to OP pesticides outdoors but they also had indoor exposure (Para occupational exposure) due to storing most of the OP pesticides in their homes.

Conclusion

8-OHdG can be a strategy to prevent the initiation or progression of inflammatory disease. With the increase of the scientific knowledge, Agri engineering must take care of a minimum exposure by the human beings so that contamination can be reduced to a maximum extent. If *Homo Sapiens* wants to be continued as species such things of genotoxicity must end in evolution.

Acknowledgement

Authors thank Geospacial public health Data Management System (GPHDMS), NRDMS, DST, Government of India for the financial assistance.

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