International Journal of Clinical Trials
Mishra BP et al. Int J Clin Trials. 2015 Aug;2(3):59-63
http://www.ijclinicaltrials.com
pISSN 2349-3240 | eISSN 2349-3259

DOI: http://dx.doi.org/10.18203/2349-3259.ijct20150593

Research Article

8-Hydroxydeoxyguanosine (8-OHdG) levels in urinary samples of pesticide sprayers on exposure to organophosphorus pesticides

B. P. Mishra[1*], Z. G. Badade[2], Bhupinder Kaur Anand[3], Jhansi Lakshmi Lingidi[1], Sapna Jaiswal[4]

[1]Department of Biochemistry, Mayo institute of Medical Sciences, Faizabad Road, Gadia, Barabanki, Uttar Pradesh, India
[2]Department of Biochemistry, MGM Medical College, MGM University of Health Sciences, Navi Mumbai, Maharashtra, India
[3]Department of Community Medicine, Career Institute of Medical Sciences & Hospital, Lucknow, Uttar Pradesh, India
[4]Department of Biochemistry, Career Institute of Medical Sciences & Hospital, Lucknow, Uttar Pradesh, India

Received: 25 April 2015
Accepted: 18 May 2015

***Correspondence:**
Dr. B. P. Mishra,
E-mail: bpmishra_72@yahoo.com

ABSTRACT

Background: Free radicals and other reactive species are constantly generated *in vivo* and cause oxidative damage to biomolecules. DNA is probably the most biologically significant target of oxidative attack. Among numerous types of oxidative DNA damage the formation of 8-hydroxyguanosine (8-OHdG) is a sensitive biomarker of oxidative stress, an adduct formed as a result of biochemical reaction between ROS and DNA. Chronic exposure to Organophosphorus (OP) pesticides is implicated in many health conditions that result from the induction of oxidative stress, including cytogenetic damage. 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.
Methods: In this study, 51 male pesticide sprayers and 39 farm workers in the age group of 18-47 years having exposure ranged from 3 to 15 years in duration were selected. The referents (n=31) were selected on the same criteria as well as they were never exposed to pesticides at any time. This study was conducted during the growing season (January, 2009 – September, 2010). The most commonly used OP pesticides like chlorpyriphos, Diazinon, Dimethioate, Monocrotofos etc., were used in this study. Urine samples from each participant were taken in sterile tubes and were stored at -20^{0}C till analysed. The concentration of 8-OHdG in samples were analyzed using ELISA.
Results: The urinary levels of 8-OHdG were found to be significantly higher in the farm workers and pesticide sprayers in contrast to the level observed in the control group ($p<0.05$). When the data was analyzed in the exposed groups in relation to duration of exposure it was found that both the farm workers and sprayers who were exposed to OP pesticides for less than 5 years showed the maximum mean values of 8-OHdG in comparison to those exposed to for more than 10 years.
Conclusions: In view of this regular bio monitoring studies in target human populations are imperative necessary due to frequent changes in pesticide formulations and introduction of newer pesticides. Despite that several life style factors may influence the urinary concentrations of 8-OHdG but still this non-invasive bio-marker 8-OHdG is preferred over other invasive techniques to evaluate the environmental and occupational exposure effect of OP pesticides on the genotoxicity of the exposed workers.

Keywords: Organophosphorus pesticides (OPP), Reactive oxygen species (ROS), Malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8OHdG)

INTRODUCTION

Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to biomolecules, a process held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged nucleic acids, proteins, and lipids. DNA is probably the most biologically significant target of oxidative attack and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age related development of the major cancers. Among numerous types of oxidative DNA damage the formation of 8-Hydroxy deoxy guanosine (8-OHdG) is a sensitive biomarker of oxidative stress. 8-OHdG, one of the oxidative DNA damage byproducts, is physiologically formed and enhanced by chemical carcinogens. Chronic exposure to Organophosphorus (OP) pesticides is implicated in many health conditions that result from the induction of oxidative stress, including cytogenetic damage.[1,2] Most widely used OP pesticides are anthropometric chemicals released into the environment for controlling agricultural pests. There are about 15000 individual compounds and 35000 formulations in use as agricultural pesticides. Though beneficial in their action, their toxicities account for a significant risk of occupational toxicity due to chronic exposure. At cellular level, pesticides have been reported to generate ROS, which catalyze increased lipid peroxidation.[3,4] ROS are removed by the endogenous antioxidant enzymes such as SOD, GSH, CAT and other peroxidases.

Among numerous types of oxidative DNA damage, the formation of 8-OHdG is a ubiquitous marker of oxidative stress. 8-OHdG, one of the oxidative DNA damage byproducts which is excreted in urine without further metabolism. The 8-OHdG is an adduct formed as a result of reaction between ROS and DNA. It establishes the link between intracellular ROS accumulation and genotoxicity. 8-OHdG if allowed to accumulate can penetrate through the DNA replication process and can retard the DNA repair mechanism.[5]

Aim of the study

The study was done to evaluate the levels of 8-OHdG in spot urinary samples in the exposed OP pesticide sprayers and farm workers engaged in mango orchards in the rural areas adjoining Lucknow, Uttar Pradesh, North India and to compare the findings obtained in these groups with that recorded in the control group belonging to similar socio-economic status having similar rural background but had no past or, current exposure to OP pesticides.

METHODS

In a cross sectional study, 51 male pesticide sprayers and 39 farm workers in the age group of 18-47 years having exposure which ranged from 3 to 15 years in duration. While selecting the subjects the care was taken that they were actively engaged in farming activity wherein they mixed and sprayed the different combinations of OP pesticides. These farmers and sprayers lived in the vicinity of the farms and also stored these pesticides in their houses. The random selection of the study group was done who did not have any chronic health conditions. The referents (n=31) were selected on the same criteria as well as they were never exposed to pesticides at any time. The background information of all participants included smoking habits, duration of smoking, types of OP pesticides used and sprayed in the farms at the time of the study. It was also noted on a pre-structured survey questionnaire, the use of any safety measures such as protective clothing, nasal masks and hand gloves. None of the farm workers on clinical examination showed sub clinical symptoms associated with occupational exposure to a mixture of OP pesticides. This study was conducted during the growing season (January, 2009 – September 2010). The most commonly used OP pesticides were chlorpyriphos, Diazinon, Dimethioate, Monocrotofos etc. Spot urine samples were selected at the morning before the starting the spraying. Urine samples from each participant were taken in sterile tubes and were stored at -20°C till analyzed. For analysis, each sample was brought to the room temperature and centrifuged at 1200 g per units and the 5 ml of supernatant was pooled. The concentration of 8-OHdG was analyzed using Enzyme Linked Immunosorbent Assay (ELISA kit; CellBio lab). It is a competitive enzyme immunoassay developed for rapid detection and quantitation of 8-OHdG in urine, serum, or other cell or tissue DNA samples. The quantity of 8-OHdG in unknown sample is determined by comparing its absorbance with that of a known 8-OHdG STANDARD curve. The kit has an 8-OHdG detection sensitive range of 100 pg/Ml to 20 ng/ml. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

Assay principle

The Oxidative DNA Damage ELISA kit is a competitive ELISA for the quantitative measurement of 8-OHdG. The unknown 8-OHdG samples or 8-OHdG standards are first added to an 8- OHdG/BSA conjugate preabsorbed ELISA plate. After a brief incubation, an anti 8-OHdG monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The 8-OHdG content in unknown samples is determined by comparison with predetermined 8-OHdG standard curve.

Statistical analysis

The data for the 2 groups were compared by the Tukey's test and significant differences between the means were determined at $p<0.05$.

RESULTS

Table 1 shows the demographic characteristics of the exposed and the control groups. The controls and the

exposed groups did not show significant differences in there mean ages. Similarly the smoking profile of the controls and the exposed groups did not defer significantly. However, the farm workers and the sprayers showed greater prevalence of alcohol consumption. The mean exposure to OP pesticides was similar in farm workers and pesticide sprayers.

Table 1: Characteristics of the agricultural workers and rural control participants.

Characteristics	Farm Workers	Pesticide Sprayers	Controls
No. of Participants	39	51	31
Average Age (Yrs)	27.6 ± 1.9	29.4 ± 1.6	26.2 ± 2.2
Period of Exposure	15.2 ± 2.9	17.6 ± 2.1	--------
Use of safety equipments	43.5	41.7	--------
Smoking Prevalence (%)	53.8	52.9	58.0
Duration of Smoking	10.2 ± 3.6	12.4 ± 2.6	10.4 ± 2.2
Bidis smoked/day	12 – 15	10 – 15	10 - 15
Alcohol (ml/day)	500 – 700	350 – 500	400 – 700

Table 2: The mean values of urinary 8-OHdG (µmol/mol creatinine) in pesticide exposed groups.

Study Group	Urinary 8 - OHdG	Urinary 8 - OHdG
	Mean ± S. E	Range
Farm workers with muscarinic symptoms (n=21)	0.589 ± 0.044	0.403 – 0.513
Farm workers with nicotinic symptoms (n=18)	0.531 ± 0.051	0.417 – 0.569
Pesticide sprayers with muscarinic symptoms (n=23)	0.539 ± 0.031	0.434 – 0.609
Pesticide sprayers with nicotinic symptoms (n=28)	0.477 ± 0.041	0.397 – 0.521

The levels of 8-OHdG in the urine samples of the participant in both the groups are shown in Table 2. A statistically significant difference was observed in the levels of urinary 8-OHdG values between the exposed and the controls was observed ($p<0.05$). The urinary levels of 8-OHdG were found to be significantly higher in the farm workers and pesticide sprayers in contrast to the level observed in the control group. However, the means values observed in the case of farm workers and pesticide sprayers did not reach the significance level. When the data was analyzed in the exposed groups in relation to duration of exposure it was found that both the farm workers and sprayers who were exposed to OP pesticides for less than 5 years showed the maximum mean values of 8-OHdG in comparison to those exposed to for more than 10 years (Table 3). In our study we also tried to analyse the data in the smokers, non-smokers, alcoholics and non-alcoholics. The results indicated no significant impact of either smoking or the alcohol on the values of 8-OHdG thereby indicating no significant role of these two factors on the levels of urinary 8-OHdG.

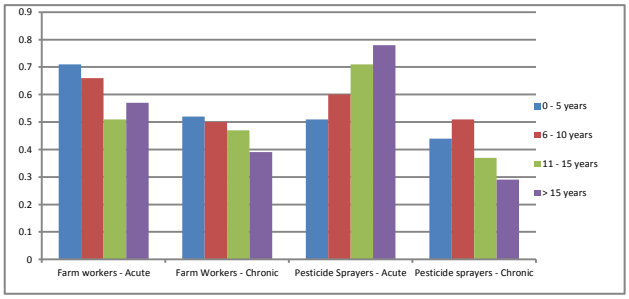

Figure 1: The mean values of urinary 8-OHdG (µmol/mol creatinine) in relation to duration of exposure.

Table 3: The mean values of urinary 8-OHdG (µmol/mol creatinine) in relation to duration of exposure.

Study Group	0 – 5 years Mean ± S. E.	6 – 10 years Mean ± S. E.	11 – 15 years Mean ± S. E.	>15 years Mean ± S. E.
Farm workers with Acute toxicity (n=18)	0.711 ± 0.071	0.663 ± 0.089	0.517 ± 0.069	0.570 ±0.055
Farm workers with Chronic toxicity (n=21)	0.522 ± 0.091	0.507 ± 0.023	0.471 ± 0.087	0.391 ± 0.011
Pesticide sprayers with Acute toxicity (n=24)	0.517 ± 0.039	0.606 ± 0.021	0.711 ± 0.099	0.783 ± 0.031
Pesticide sprayers with Chronic toxicity (n=27)	0.441 ± 0.021	0.511 ± 0.026	0.375 ± 0.013	0.299 ± 0.071

DISCUSSION

The present results indicate that there is statistically significant differences in the urinary levels of 8-OHdG in the exposed workers and the control group, thereby indicating oxidative stress and DNA damage and that the amount of DNA damage co-related with the extent of pesticide exposure. Some studies have suggested that the oxidative stress and the extent of pesticide exposure. The previous studies have also suggested that the oxidative stress and the DNA damage are common mechanisms by which OP pesticides disrupt the function of human cells.

Oxidative DNA damage reportedly plays an important role in a number of pathological conditions including carcinogenesis. However, few epidemiological studies have reported the usefulness of measuring the bio-markers of oxidative stress (8-OHdG) and DNA damage. Lagorio and colleagues (1994) reported a dose response effect between the occupational exposure to OP pesticides and urinary levels 8-OHdG.In our study, we did not find the influence of smoking and consumption of alcohol on the levels of 8-OHdG.[6] Loft and Poulsen (1996)[7] reported the correlation between the body mass index (BMI) and the levels of 8-OHdG. They observed that the leanness is reported to be associated with increased excretion of 8-OHdG possibly due to the influence of a higher metabolic rate. Similar findings were observed in our study also where the study group with lower BMI had a higher excretion of urinary 8-OHdG. Similarly the role of diet also set to influence the levels of excretion of 8-OHdG but we failed to observe any difference between the levels of urinary 8-OHdG between the vegetarians and non-vegetarian workers.[8]

Both OP pesticides and farm workers had higher levels of 8-OHdG then that in the controls. Previous studies[9,19] used 8-OHdG as a marker of oxidative damage used a 24 hour urine collection, but in our study only spot urines were feasible as these samples contain adequate levels of 8-OHdG for measurement in the exposed workers.[10,11] However, recent studies indicated that the urinary levels of 8-OHdG can vary over a 24 hour period when measured for consecutive days.[12-14] Therefore it is suggested that the single values of 8-OHdG should be considered with caution, and when spot urines are used. Oxidative DNA lesions generally appear within hours after exposure.[15] Mutagenic chemicals and there persistence may be briefed as indicated by the diminished mutagenicity within 24 hour or less after exposure.[1,16,17] It may be that the urinary levels of 8-OHdG reflect short term exposures compared to the other markers examined in the serum (MDA) of agricultural workers thereby reflecting more current exposures to OP pesticides.[2,18]

CONCLUSIONS

The urinary 8-OHdG is an adduct formed as a result of reaction between ROS and DNA. It establishes a link between intracellular ROS accumulation and genotoxicity. In view of this regular bio monitoring studies in target human populations are imperative necessary due to frequent changes in pesticide formulations and introduction of newer pesticides. Despite that several life style factors may influence the urinary concentrations of 8-OHdG but still this non-invasive bio-marker 8-OHdG is preferred over other invasive techniques to evaluate the environmental and occupational exposure effect of OP pesticides on the genotoxicity of the exposed workers.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Mishra BP, Badade ZG, Anand BK, Lingidi JL, Jaiswal S. 8-Hydroxydeoxyguanosine (8-OHdG) levels in urinary samples of pesticide sprayers on exposure to organophosphorus pesticides. Int J Clin Trials 2015;2(3):59-63.