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Evaluation of Genetic Damage in Farmers Exposed to Pesticide Mixtures

Abhay Singh Yadav and Gulshan Sehrawat

Human Genetics Laboratory, Department of Zoology, Kurukshetra University, Kurukshetra 136 119, Haryana, India

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ABSTRACT Environment surrounding us is being polluted day by day by various kinds of chemicals and xenobiotics. Pesticides are one such group, which are toxic in nature yet indispensable as they are used in variety of human activities such as agriculture, aquaculture and household tasks. Excessive dependency on these chemicals is a serious concern today. For the present investigation, a total of 62 individuals including 33 pesticide users (exposed) and 29 non-users (controls) gave blood samples. Comet assay being a highly sensitive and low cost technique was used to access the level of genetic damage in exposed population. Hundred cells were analysed from each individual and Damage Index (DI) was calculated using various comet parameters such as comet length, tail length, tail area, percentage DNA in tail, tail moment and olive tail moment. The mean duration of exposure to pesticides in farmers was 14.032 years. The mean value of comet length was 94.96±4.22 in exposed cases as compared to 36.56±2.11 in controls. The mean value of tail length was found to be 52.18±3.74 and 7.01±1.47 in exposed and controls, respectively. The mean value of percentage of DNA in tail in exposed and controls was 27.45±1.64, 9.04±0.67, respectively. The mean tail area was 19.23±4.75 in exposed and 1.39±0.32 in control individuals. The mean tail moment and olive tail moment were found to be 16.91±2.14, 15.58±9.07 in exposed and 1.04±0.032, 1.82±0.32 in case of control individuals. All these comet parameters were found to be statistically significant at 0.005 level using t-test. The percentage of DNA in tail was also found to increase with increase in duration of exposure.

INTRODUCTION

Pesticides hold an indispensable place in Indian agriculture as well as several household tasks, but their indiscriminate use is giving rise to various health related problems. Almost every system of human body is affected by these chemicals, whether respiratory, reproductive, nervous or circulatory. There are four main groups into which these chemicals have been classified, viz. Organophosphates, Organochlorines, Carbamates and Pyrethroids. As majority of these chemicals are non- biodegradable, they tend to accumulate in environment and also enter into the food chain.

The most potent and important chemical class is of organophosphates, which act by inhibiting acetylcholinesterase hydrolysis of acetylcholine, resulting in acetylcholine accumulation in neuromuscular synapses. The acute toxic effects of organophosphate pesticides are due to the hyperstimulation of muscarinic and nicotinic receptors, resulting in symptoms that range from increased secretions to death by respiratory depression (Alessandra Fortes Aiub et al. 2002). Organophosphate-based pesticides have been associated with pathological and chromosomal damage in humans. There are also epidemiological links with cancer (Webster et al. 2002). Occupational exposure to pesticides would increase BCL2-IGH prevalence and the frequency of BCL2-IGH bearing cells especially during the high pesticide use period. Increasing incidence of non-Hodgkin's lymphoma have been associated repeatedly with farming occupation and particular attention focused on the role of pesticide exposure explained this trend (Roulland et al. 2004).

Pesticides possibly induce oxidative stress leading to the generation of free radicals and alteration in antioxidant/free radical scavenging enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutothione transferase (Ahmed et al. 2000). There is substantial experimental and epidemiological evidence that many pesticides in widespread use around the world are immunosuppressive. Numerous animal studies have shown a variety of effects of pesticides on the immune system, including decreased antibody formation by 70% after exposure to pesticides

Corresponding Author:
Prof. Dr. Abhay Singh Yadav
Chairman, Department of Zoology,
Kurukshetra University, Kurukshetra, 136119,
Haryana.
Telephone: +919416173289
E-mail: abyzkuk@gmail.com

such as Captan, Lindane and Malathion; decreased cell mediated immunity is also indicated (Xavier et al. 2004).

During the last few years, there has been a great interest in developing rapid and simple tests to identify the effects of exposure to environmental agents that can affect the health of individuals due to DNA damage. One of these methods is the comet assay, which is a rapid and sensitive technique to measure sites sensitive to basic pH and DNA breaks in individual cells (Martino-Roth et al. 2003). The comet assay is sensitive to damage above about 50 breaks per diploid mammalian cell and will lose sensitivity above about 10,000 breaks per cell (Olive and Banath 2006). Ostling and Johanson (1984) were the first to develop a microgel electrophoresis technique for detecting DNA damage at the level of the single cell. In their technique, cells embedded in agarose were placed on a microscopic slide, the cells were lysed by detergents and high salt and the liberated DNA electrophoresed under neutral conditions. Cells with an increased frequency of DNA double strand breaks (DSBs) displayed increased migration of DNA towards the anode. Subsequently, Singh et al. (1988) introduced a microgel technique involving electrophoresis under alkaline conditions for detecting DNA damage in single cells. Since then, the applications and the number of investigators using this technique have increased almost exponentially (Tice et al. 2000).

The advantages of comet assay include the applicability to various tissues and special cell types, sensitivity for detecting low levels of DNA damage, requirement for small number of cells per sample, general ease of test performance, the short time needed to complete a study and relatively low cost (Brender-Schwaab et al. 2005).

MATERIALS AND METHODS

Materials: During the present study farmers exposed to pesticides were investigated and healthy individuals selected at random were taken as controls. A total of 62 subjects were investigated, out of these 33 were pesticide users and 29 were controls. The study was approved by Institutional Ethics Committee of Kurukshetra University, Kurukshetra. Informed consent was obtained from the individuals included in the study. Table 3 shows the list of most used pesticides by the farmers in the area of investigation. The extensively used chemical class was of Organophosphates.

Blood Samples: Venous heparinized blood samples (1ml) were drawn from each exposed subject donor and matched control donor. These were processed for analysis at the Human Cytogenetics Laboratory, Department of Zoology, Kurukshetra University, Kurukshetra.

Laboratory Methods: Single Cell Gel Electrophoresis (SCGE) or Comet Assay was used in the present study. The protocol of Ahuja and Saran (1999) was followed.

Statistical Analysis: Statistical analysis was done using t-test with the help of SPSS 11.5.

RESULTS

The main characteristics of the exposed and matched controls are given in Table 1. A total of 62 subjects have been studied out of which 33 were exposed to pesticides and 29 served as controls. Out of these 45 (72.58%) were males and 17 (27.41%) were females. The mean duration of exposure to pesticides was 14.0328 years.

Table 1: General characteristics of exposed and control groups

S. No.	*Variables*		*Exposed*	*Control*
1.	n		33	29
2.	Average age (yrs.)		38.15	30.55
3.	Sex	Males	33	12
		Females	-	17
4.	Smoking habits	Non-smokers	16	29
		Smokers	17	-
5.	Drinking habits	Non-alcoholic	24	29
		Alcoholic	9	-
6.	Dietary habits	Non-vegetarian	4	2
		Vegetarian	29	27

The various comet parameters taken for the investigation were comet length, tail length, percentage of DNA in tail, tail area, tail moment, olive tail moment. All these parameters were found to be statistically significant at 0.005 level (Table 2). The mean value of comet length in exposed group was 94.96±4.22, whereas in control group it was 36.56±2.11. Mean tail length was 52.18±3.74 in exposed and 7.01±1.47 in control group. The mean percentage of DNA in tail was found to be 27.45±1.64 in exposed individuals as compared to 9.04±0.67 in controls. Mean tail area was 19.23±4.75 in exposed and 1.39±0.32 in control group. Mean tail moment

and mean olive tail moment were 16.91±2.14, 15.58±1.57 in exposed group and 1.04±0.32, 1.82±0.32 in control group, respectively.

Table 2: Comet assay parameters in peripheral blood lymphocytes of control group and the exposed farmers

S. No.	*Comet parameters*	*Subjects*	*Mean ± S.E.*	*S.D (±)*
1.	Comet length	controls	36.56±2.11	11.38
		exposed	94.96±4.22	24.24
2.	Tail length	controls	7.01±1.47	7.92
		exposed	52.18±3.74	21.53
3.	% DNA in tail	controls	9.04±0.67	3.64
		exposed	27.45±1.64	9.42
4.	Tail area	controls	1.39±0.32	1.72
		exposed	19.23±4.75	27.28
5.	Tail moment	controls	1.04±0.32	1.76
		exposed	16.91±2.14	12.34
6.	Olive tail moment	controls	1.82±0.32	1.74
		exposed	15.58±1.57	9.07

Table 3: Most used pesticides among farmers in the area of investigation with their respective chemical class and WHO classification

S. No.	*Name of pesticides*	*WHO classification of toxicity*	*Chemical class*
1.	Phorate	Class I A	Organophosphate
2.	Endosulphan	Class II	Organochlorine
3.	Glyphosate	Table V	Organophosphate
4.	Dimethoate	Class II	Organophosphate
5.	Chloropyriphos	Class II	Organophosphate

Class I A- Extremely hazardous
Class II- Moderately hazardous
Table V- Pesticide unlikely to present acute hazard in normal use

The percentage of DNA in tail in control and exposed individuals in relation to duration of exposure is shown in Figure 1. The figure shows that with increase in duration of exposure the percentage of DNA in tail also increased. In case of controls the value of percentage DNA in tail was 10.65, whereas, it increased from 24.17 to 33.94 in exposed individuals from up to 15 years of exposure to above 15 years of exposure, respectively.

DISCUSSION

The present study was performed using the comet assay to evaluate DNA damage if any, in farmers handling and spraying various pesticide mixtures. Our analysis consisted of an exposed population of farmers along with an unexposed control group. In this study we found out significant increase in DNA damage in terms of various comet parameters. The most used chemical class was of organophosphates and the prominent one was Phorate.

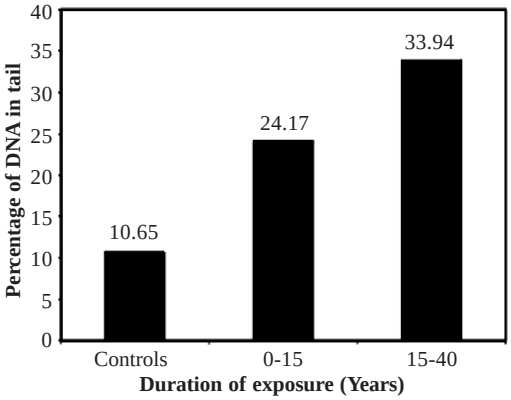

Fig. 1. The percentage DNA in tail in control and exposed individuals in relation to duration of exposure

The comet parameters we took for this study were the comet length, tail length, tail area, % DNA in tail, tail moment and olive tail moment. All values were found to be higher in case of exposed individuals as compared to controls. Studies by Grover et al. (2003), also revealed similar findings that occupational exposure during pesticide production to mixture of pesticides induces a significant increase in the level of DNA damage and that exposed workers had a significant greater mean DNA tail length than controls. Studies on comet tail length done by Das et al. (2008) showed that tail length increased from 0.11μm to 0.95μm with the increase in concentration of Acephate from 0 to 7μM. Studies done on floriculturists by Castillo-Cadena et al. (2006) also showed that DNA damage in floriculturist population was 22.9% higher as compared to controls.

Persistent and prolonged use of pesticides is also associated with several human diseases both acute and chronic, including many types of neoplasias. Studies performed by Roulland et al. (2004) have shown that prevalence of BCL2-IGH translocation in farmers occupationally exposed to pesticides was 71%. The most potent class among these pesticides is of organophosphates, which have long term effect on the nervous system, as they inhibit acetyl cholinesterase in a range of nerve, neuromuscular and glandular tissues where this enzyme plays a key role in cell to cell communication as revealed by Karalliedde

et al. (2003). Studies with organophosphate Malathion on a freshwater teleost using the comet assay technique. Kumar et al. (2010) suggested higher DNA damage at all concentrations and exposure time duration. Reus et al. (2008) revealed that after acute as well as chronic treatment with Malathion, DNA damage increased in the total blood of rats.

In our study we also found that with increasing duration of exposure the percentage DNA in tail also increased. Also, increase in genotoxic effects in workers exposed to pesticides with increase in duration of exposure has been reported by Grover et al. (2003). Experiments performed by Alessandra Fortes Aiub et al. (2002) using comet assay identified that Temephos produced dose-dependent severe (Type IV) lesions in the DNA of total blood cells of Wistar rats. Higher DNA damage was found in various comet parameters in different studies performed by Jors et al. (2007) and Undeger and Basaran (2005). Damage index and damage frequency observed in the exposed group were significantly higher in relation to the controls in studies performed by Carla et al. (2009) and Simoniello et al. (2008). DNA migration increases in human lymphocyte cell cultures as a result of treatment with Cypermethrin, 2, 4-D and Chloropyrifos at high concentrations (Sandal and Yilmaz 2010).

Thus, pesticide mixtures have genotoxic effects on human beings, but we can not pinpoint on one particular compound, which specifically causes genetic damage. Still we can do efforts to minimize harmful effects on farmers engaged in pesticides use by spreading awareness through local bodies, make them use all possible protective gears and replacing chemical pesticides with bio-pesticides whenever and wherever possible.

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