

Risk Assessment of Occupational Exposure to Pesticides among Pesticide Distributors of Punjab (India) Using Single Cell Gel Electrophoresis

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ABSTRACT Punjab being the major state of India that utilizes pesticides comprises different populations, which are directly or indirectly exposed to the pesticides, mainly the pesticides manufacturers, formulators and distributors. Amongst these pesticide distributors are least affected considered despite their continuous exposure to them. The present study was undertaken to investigate the occurrence of genotoxic effects in pesticide distributors from three cities in Punjab. The methods used included Comet Assay to assess DNA damage. The results showed that a significant increase in the frequency of DNA damage was found. An increasing trend in genetic damage was observed between the workers with increasing years of exposure as revealed by an ANOVA test. Similarly, the effect of other confounding factors such as age, diet and alcohol drinking habits were also studied. Conclusively, the use of protective measures and other safety regulations is emphasized among the pesticide distributors to prevent further exposure to this group.

INTRODUCTION

Pesticides are among the most extensively used industrial chemicals in the world today and as they were specifically designed to be toxic, they are also among the most hazardous compounds to human health (Tsimbiri et al. 2015). Human populations are unavoidably exposed to environmental pollutants in physical, chemical or biological forms through products presented or degraded in air, water, soil or food (Gomez-Arroyo et al. 2000). A growing number of well-designed epidemiological and molecular studies provide substantial evidence that the pesticides used in agricultural, commercial and household applications are associated with cancer risk. The occupational toxic risks from agricultural activities affect human and environmental health (Mejia et al. 2014). This risk is associated both with those applying the pesticides and under some conditions, those who are simply bystanders to the application (Alvanja et al. 2013). Occupational exposure to pesticides is concerned mainly with pesticide distributors,

formulators, loaders, applicators, bystanders and rural field workers. Assessing and managing the occupational health risks posed by the use of pesticides on the population directly or indirectly exposed to pesticides is a complex but essential task for occupational health specialists and toxicologists (Maroni et al. 2006). Among the possible effects related to this exposure, genetic damage has important health implications as an induction of various types of cancers (Mink et al. 2008; Alvanza and Bonner 2012; Michael et al. 2013).

Therefore, the health hazards caused by pesticides in humans are of special interest. Genetic bio monitoring of populations exposed to potential carcinogens is an early warning system for genetic diseases or cancer. It also allows identification of risk factors at a time when control measures could still be implemented (Kassie et al. 2000; Johnson et al. 2014; Bernardi et al. 2015). The comet assay permits quantitative assessment of the effects of DNA damage or apoptosis. Any DNA damage is represented as the tail length of the DNA. The damage usually detected by comet assay is single strand breaks and double strand breaks (Mozaffarieh et al. 2008; Enciso et al. 2009; Cortes-Gutierrez et al. 2012; Bajpayee et al. 2013; Karbaschi and Cooke 2014; Narmadha et al. 2014; Ibero-Baraiber et al. 2015; Patil et al. 2015).

Punjab is one of the states of India with high pesticide consumption. Amongst various ex-

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posed populations, pesticide distributors are an important link in the pesticide distribution chain in Punjab and other parts of country. Distributors (especially workers) are in direct contact with pesticides as they spend at least 4hour per day in a closed environment and even eat, drink and smoke in the same workplace. Loading and unloading of packets from the cartons and its transport from the ware-house and show-room is done using bare hands and in case of heavy loads workers carry them on shoulders or the head. Spillage of the pesticides is quite obvious during the loading and unloading process, which is directly or indirectly affecting the health of the exposed population.

Objectives

As there is no literature available with respect to health problems among these pesticide distributors, the present investigation has been undertaken to reveal the level of genotoxicity in pesticide distributors of Punjab using comet assay. On the other hand, the effect of confounding factors like age, diet and alcohol drinking habits is also studied.

MATERIAL AND METHODS

Blood samples were collected from 50 pesticide distributors from three different cities in Punjab namely, Amritsar, Jalandhar and Jandi-ala. For the control group, a set of 50 individuals was selected from these three cities from the same sex, age and socioeconomic status as that of the exposed group. However, these individuals did not have any kind of direct pesticide exposure and mainly were working in closed offices for 8 hours per day. Also it was considered that they should not have any history of X ray exposure or antibiotic therapy three months before the sampling period. Information about personal, medical and exposure histories from each subject (both control and exposed population) was recorded on a predesigned questionnaire.

The alkaline SCGE assay (Ahuja and Saran 1999) was carried out as a test to assess genetic damage in pesticide distributors. The blood samples (100-200 μ l) were collected from fingertip punctures in heparinized eppendroff tubes. The samples were transported to the laboratory in an icebox and processed within 3-4 hours for the SCGE assay.

The slides were coded and a cell scoring was done under a binocular microscope with the help of ocular micrometer. 100 cells per individual were scored for tail length and frequency of damage. The calibration factor of an ocular micrometer was first calculated as 2.5mm using stage micrometer. Firstly, the scoring was done under low magnification (10x) and then under high magnification (40x). The comet tail length of the DNA of each cell measured by an ocular micrometer was multiplied by the calibration factor of an ocular micrometer to give the exact measurement of the DNA migration length. Half of the diameter of the comet head was subtracted from the DNA migration length to give the quantitative value for DNA damage. No scoring was done near the edges of the gel. Photomicrography of normal cells and cells with comet was taken using a camera (Olympus 3X) fitted on to a trinocular microscope (Olympus CX21) and the photographic prints were obtained. The results were statistically analyzed using the analysis of variance (ANOVA) and student's t-test.

RESULTS

The results of the present investigation are summarized in Tables 1-4 and Figures 1-2. Subjects studied (control and exposed) were males only. The mean age of the exposed population was 41.02 ± 1.64 years (20-60 years) and of the control group was 41.34 ± 1.55 years (20-60 years). There were 12 (24%) vegetarians and 38 (76%) non-vegetarians in the exposed group, and 31 (62%) vegetarians and 19 (38%) non-vegetarians in the control group. The exposed group involved 33 (66%) alcoholics and 17 (34%) non-alcoholics, whereas the control group comprised of 6 (12%) alcoholics and 44 (88%) non-alcoholics. The duration of pesticide exposure varied from 1-40 years in the exposed group with an average of 16.26 ± 1.57 years. Protective measures were not used by most of the exposed population despite the fact that they were aware of the pesticide's toxicity. Only 2 (4.0%) out of 50 subjects were found to use some sort of gloves and mask during the handling of pesticides.

A comparative analysis of comet assay end points between exposed and control group is represented in Table 1. Exposed workers showed significantly greater comet tail length (as shown in Fig. 1) and the mean frequency of damaged cells in leucocytes than the control group. The

values were $t=11.13$ ($p<0.01$) and 20.20 ($p<0.01$), respectively for the comet tail length and frequency of damaged cells.

Table 1: Comet assay end points in controls and pesticide distributors

Study groups	Tail length (μm , mean \pm S.E)	Frequency of damaged cells (%, mean \pm S.E)
Control (n=50)	15.89 \pm 0.39	32.52 \pm 1.28
Exposed (n=50)	26.27 \pm 0.83	71.90 \pm 1.52
t-value	11.13**	20.20**

**Significant at 1%, ($p<0.01$)

For analyzing the effect of duration of pesticide exposure, the exposed population was di-

vided into four groups, that is having 01-10, 11-20, 21-30 and 31-40 years of exposure as given in Table 2. The analysis of variance (ANOVA) revealed an increasing trend in genetic damage between workers with increasing years of pesticide exposure (comet tail: $F=37.39$, $p<0.01$ and damaged cell frequency: $F=5.13$ $p<0.01$).

The effect of diet (vegetarian versus non-vegetarian) on the values of genetic parameters assessed has been given in Table 3. It was revealed that the values of comet tail as well as frequency of damaged cells showed increased values in case of non-vegetarians as compared to control except in case of frequency of damaged cells in controls. This difference between values of both the parameters in vegetarians and non-vegetarians is found to be statistically in-

Table 2: Comet tail length and frequency of damaged cells in exposed individuals according to duration of pesticide exposure in pesticide distributors

Study groups	Tail length (μm , mean \pm S.E)	Frequency of damaged cells (% mean \pm S.E)	t-values	
			Comet Tail	Damaged frequency
Years of pesticide exposure				
(I) 01-10 (n=21)	21.47 \pm 0.46	66.57 \pm 2.58	I-II	5.00**
(II) 11-20 (n=16)	26.73 \pm 0.94	73.88 \pm 2.50	I-III	3.00**
(III) 21-30 (n=06)	30.46 \pm 0.79	76.83 \pm 2.61	I-III	0.75 N.S
(IV) 31-40 (n=07)	34.36 \pm 1.61	81.14 \pm 0.96	I-III	2.02 N.S
			I-IV	10.69**
			I-IV	3.20**
			II-IV	4.29**
			II-IV	2.12*
			III-IV	2.00 N.S
			III-IV	1.64 N.S
F-value	37.39**	5.13**		

** =Significant at 1%; * = Significant at 5%; NS =Non significant

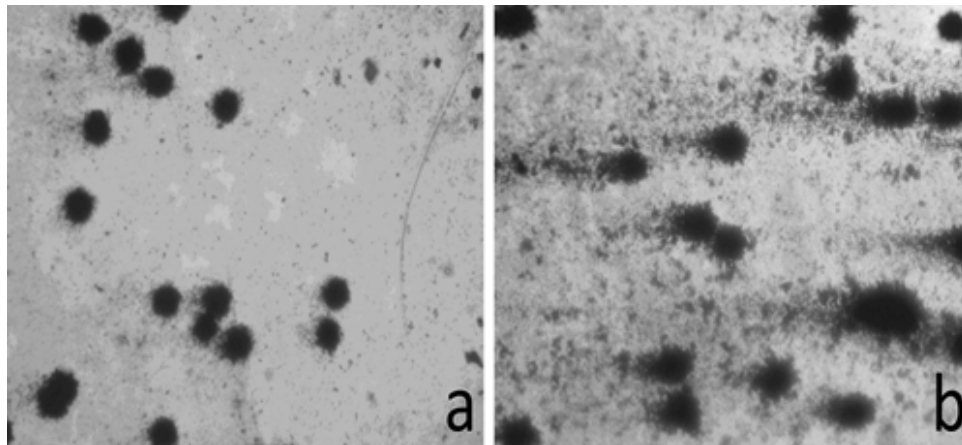


Fig. 1. (a) Comet assay in blood leukocytes showing undamaged cells (b) Highly damaged cells with long tails

Table 3: Mean comet tail length (μm) and frequency of damaged cells in leucocytes of controls and pesticide distributors according to diet

Parameter	Group	Control	Exposed
Comet tail (μm , mean \pm S.E)	Vegetarians	15.64 \pm 0.53 (n=31)	22.75 \pm 0.93 (n=12)
	Non- vegetarians	16.32 \pm 0.58 (n=19)	27.38 \pm 0.09* (n=38)
Frequency of damaged cells (% , mean \pm S.E)	Vegetarians	33.7 \pm 1.59 (n=31)	69.17 \pm 2.65 (n=12)
	Non- vegetarians	30.58 \pm 0.58 (n=19)	73.38 \pm 1.68 (n=38)

* = significant at 5

significant except for the comet tail length where in the value is significantly higher in non-vegetarians as compared to vegetarians ($t=2.51$, $p<0.05$).

The effect of alcohol consumption on the values of DNA damage parameters is given in Table 4. It was examined that the mean values of the comet tail length and frequency of damaged cells were higher in alcoholics as compared to non-alcoholics for both control and exposed groups. However, the difference was statistically insignificant except for the value of comet tail length in controls, which is significantly higher

in case of alcoholics as compared to non-alcoholics. The values were 18.43 \pm 0.91 for alcoholics and 15.56 \pm 0.41 for non-alcoholics ($t= 4.60$, $p<0.01$).

The age of the individual may also have affected the occurrence of genetic damage in the studied population. For this purpose, the control and exposed subjects were divided into four age groups, that is 20-30, 31-40, 41-50 and 51-60 years. The mean values of comet tail length and mean frequency of percentage damaged cells in different age groups are given in Figures 2 (a) and (b).

Table 4: Mean comet tail length (μm) and frequency of damaged cells in leucocytes of control population and pesticide distributors according to alcohol habits

Parameter	Group	Control	Exposed
Comet tail (μm , mean \pm S.E)	Alcoholic	18.43 \pm 0.91** (n=6)	26.65 \pm 1.03 (n=33)
	Non-alcoholic	15.56 \pm 0.41 (n=44)	24.22 \pm 1.16 (n=17)
Frequency of damaged cells (% , mean \pm S.E)	Alcoholic	34.67 \pm 3.27 (n=6)	72.33 \pm 1.84 (n=33)
	Non-alcoholic	32.23 \pm 1.39 (n=44)	68.06 \pm 0.60 (n=17)

**=significant at 1%

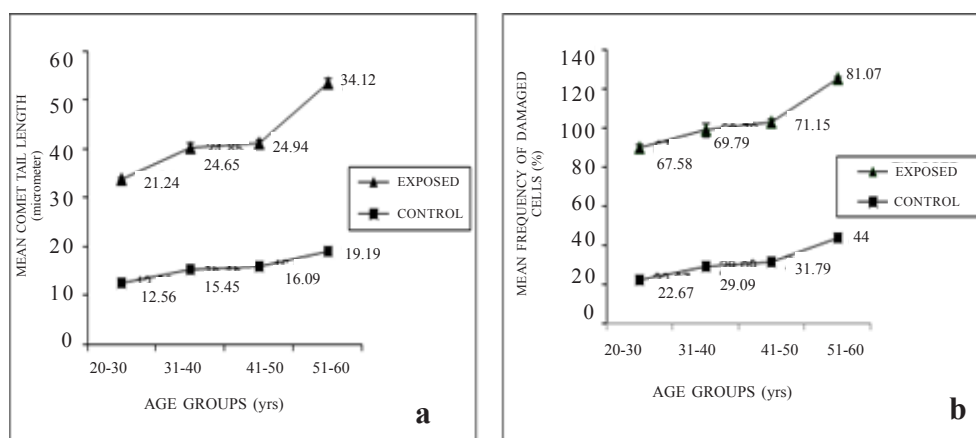


Fig. 2. (a) Migration of DNA (b) Mean frequency of damaged cells in blood leucocytes with respect to the age in controls and pesticide distributors

The value of both the end points studied increased with an increase in the age of an individual. The significant increasing trend in genetic damage was found in control and exposed population groups with increasing age (comet tail: $F=46.71$, $p<0.01$ and damaged frequency $F=39.64$, $p<0.01$ for control group and comet tail: $F=10.27$, $p<0.01$, and damaged frequency $F=44.60$, $p<0.01$ for exposed group).

DISCUSSION

The present study was undertaken to evaluate the genetic damage in pesticide distributors of Punjab. The results of the present investigation indicated that occupational exposure to a mixture of pesticides induces a significant increase in the level of DNA damage. Earlier, the several groups of workers exposed to a mixture of pesticides were investigated by various scientists with different selection criterions (Garaj-Vrhovac and Zeljezic 2000; Garaj-Vrhovac and Zeljezic 2001; Moretti et al. 2002; Undeger and Basaran 2002; Grover et al 2003; Bhalli et al. 2006; Kehdy et al. 2007; Silva et al. 2008; Chadha et al. 2012; Benedetti et al. 2013; Chadha and Prabha 2013).

Many factors have been reported to produce effects on the values of genetic markers, namely age, exposure, diet, gender, smoking and season (Moller et al. 2000). For the evaluation of the effect of duration of pesticide exposure, the entire population was divided into four subgroups ranging from 1-40 years and an increasing trend has been found with increase in duration of exposure. A number of earlier studies have reported a positive relationship between genetic damage and duration of exposure (Lindahl 1993; Garaj-Vrhovac and Zeljezic 2001; Moretti et al. 2002; Undeger and Basaran 2002; Singh et al. 2013). On the other hand, some contradictory findings to the results have also been found reporting no significant association between the DNA damage and duration of exposure (Bohar et al. 1989; Bolognesi et al. 2002; Moretti et al. 2002; Sailja et al. 2006; Costa et al. 2007; Silva et al. 2008). Studies by Yadav and Sherawat (2011) also revealed similar findings that the percentage of DNA in the tail was also found to increase with an increase in the duration of exposure.

For the assessment of the effect of age on the value of genetic markers the pesticide distributors and control population were divided into four groups. Age has found to have signif-

icant effect on the extent of DNA damage. The results demonstrated that aging tended to increase the level of DNA damage in vulnerable cells. The level of DNA damage and its repair capacity have been widely considered factors closely related to aging and cancer (Bohar et al. 1989). Few studies have reported a significant association between genetic damage with increase in age (Bohar et al. 1989; Lindahl 1993; Moller et al. 2000; Bolognesi et al. 2002; Sailja et al. 2006; Costa et al. 2007; Chadha and Yadav 2011; Chadha et al. 2013). According to some DNA related theories of aging, cells from older individuals are expected to have increased levels of basal DNA damage, possibly accompanied by a reduced rate of damage recognition and repair (Lindahl 1993). DNA damage can account for genetic changes that occur at the different stages in the progression from anaplastic growth to metastasis and therefore, dietary factors that reduce the impact of free radicals are likely to protect against cancer (Benkovic 2009; Mehta et al. 2010; Azevedo et al; 2011; Han et al. 2011; Bernardi et al. 2015).

In the present study, the diet did not show a significant relation with genetic damage. Similar results have been suggested by some other studies (Dhawan et al. 2001; Pastor et al. 2002). The mean values of non-vegetarians were higher than vegetarians for both the study groups. It can be assumed that the diet of workers, which comprises large amounts of natural food and vegetables, may reduce the clastogenic effects of toxicants. Various experimental studies indicated that a diet rich in fruits and vegetables is indeed associated with low incidence of various cancers (Hoyos et al. 1996). This prediction is mainly attributed to dietary contents of antioxidants such as vitamin E, C and various carotenoids. When the effect of alcohol was examined for genotoxic damage, no positive association was found between alcoholics and non-alcoholics of the exposed population. However, significant difference was found for comet tails between alcoholics and non-alcoholics of the control population (Suralles et al. 1997; Bloching et al. 2000; Gomez-Arroyo et al. 2000; Sailja et al. 2006; Mc Cauley et al. 2008).

In the present study, the researchers found a positive and significant increase in the damage due to pesticide exposure as the pesticide distributors, specifically workers dealing with loading and direct handling of pesticides, are

prone to direct exposure to pesticides for several hours a day. The exposure occurs through ingestion, inhalation and dermal contact. Factors like non-use of personal protection equipment, work practices related to hygiene, spills, and attitudes towards risk may all influence the degree of pesticide exposure and can be incorporated into exposure estimates but the relationship of these factors to exposure is complex (Kamel and Hoppin 2004 and Kesavchandran et al. 2010).

CONCLUSION

The positive findings of increased genetic damage in the blood leukocytes of pesticide distributors in the present work indicate the potential genetic hazards posed by excessive use of pesticides in Punjab, particularly in Amritsar, Jalandhar and Jandiala.

RECOMMENDATIONS

The use of protective measures and other safety regulations should be emphasized among the pesticide distributors to prevent further exposure. Furthermore, it is very important that surveillance should be maintained for the high-risk group, which has an increased risk for genetic damage.

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