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Evaluation of Genotoxicity in Pesticide Distributors of Punjab

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ABSTRACT Since before 2000 BC humans have utilized pesticides to protect their crops. From that time the pesticides have become an important tool as a plant protection agent for boosting food production. Punjab is a major zone of consumption of pesticides in India and therefore this state has a large group of population which is exposed directly or indirectly to pesticides. Amongst these the workers involved in the pesticide distribution are least considered. So the present study has been undertaken for investigating 50 pesticide distributors from different cities of Punjab and similar number of control individuals using micronuclei assay in buccal epithelial cells. Significant increase in the frequency of micro nucleated and binucleated cells was found in pesticide distributors as compared to controls. Increasing trend in genetic damage was observed between the workers with increasing years of exposure as revealed by ANOVA. The effect of age, diet and alcohol drinking habits was also studied.

INTRODUCTION

Agricultural development continues to remain the most important objective of Indian planning and policy. In the process of development of agriculture, pesticides have become an important tool as a plant protection agent for boosting food production. Further, pesticides play a significant role by preventing many dreadful diseases. Since before 2000 BC, human have utilized pesticides to protect their crops. During 1940s manufacturers began to produce large amounts of synthetic pesticides and their use became widespread (Daly et al. 1998).

Pesticide use has increased 50-fold since 1950 and 2.3 million tones of industrial pesticides are now used each year (Miller 2002). Today, the Indian pesticide industry comprises more than 500 pesticide formulations. The pesticide formulations produced in the country are mainly of the conventional type. The use of pesticides is high in few parts of the country such as Andhra Pradesh, Karnataka, Maharashtra, Gujarat and Punjab.

Punjab is one of India's prosperous states. This prosperity has been largely due to its success in the agricultural green revolution It is major zone of consumption of pesticides in India, therefore this state has a large group of population which is exposed to pesticides directly or indirectly viz. workers in pesticides manufacturing industry, pesticide shopkeepers, agricultural workers and workers engaged in other public health activities. A number of recent studies are coming with the health implications of pesticide workers and susceptible general population in India (Dewan et al. 2004; Ahmed et al. 2006; Singh and Ubikrishnan 2006; Singh et al. 2007). It is evident that Punjab is trapped into a disastrous vicious cycle of slow poisoning. Assessment of genotoxic effects of pesticides in occupationally exposed subjects may be used as fairly reliable biomarkers of early biological alterations (Garaj-Vrhovac and Zeljezic 2001). DNA alterations are known to be indicators of early damage in the effected organisms and can be considered as effective and beneficial strategy for risk management. A number of studies have examined the genotoxic effects in pesticide industry workers (Garaj-Vrhovac and Zeljezic 1999; Padmavathi et al. 2000; Garaj-Vrhovac and Zeljezic 2000, 2001; Grover et al. 2003; Bhalli et al. 2006). Pesticide distributors are an important link in pesticide distribution chain in Punjab and other parts of country. Distributors (especially workers) are also in direct contact with pesticides as they spend at least 4hrs/day in closed environment and even eat, drink and smoke in the same workplace. They don't wash hands after each sale of pesticide to customers. Moreover, loading and unloading of packets from the cartons and its transport from the ware house and show room is done by bare hands and in case of heavy loads workers carry them on shoulders or the head. Spillage of the pesticides is quite obvious during loading and unloading process. It was seen that there is no literature available with respect

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to health problems among these pesticide distributors.

Objectives

The main aim of the study was to asses the genotoxic effects in pesticide distributors of Punjab using micronucleated buccal mucosa cells as parameter.

MATERIAL AND METHODS

During the entire course of study the 50 individuals occupationally exposed to pesticides, that is, pesticide distributors and similar number of controls having same sex, age and socio-economic status were investigated. Most of the pesticide distributors belonged to Amritsar, Jalandhar and Jandiala. Information about personal, medical and exposure histories from each subject was recorded on a pre-designed questionnaire. Attention was paid to exclude any individual who had been subjected to X-ray treatment or antibiotic therapy three months prior to when samples were taken.

Exfoliated cells were collected from buccal mucosa by swabbing with a spatula. Slides were processed using the method of Yadav and Chadha (2002) with minor modification. Buccal smear on glass slides were transported to laboratory within 3-4 hr of sample fixation. Buccal smear preparation was hydrolyzed in 1 N HCl at 60°C for 8 minutes followed by a rinse in tap water. Then slides were stained in aceto-orcein and then a brief washing in ethanol and distilled water was given. Slides were air dried and coded. At least 1000 cells were scored and criteria of Tolbert et al. (1992) was followed for scanning of micronuclei and along with it the frequency of binucleated cells was also calculated. The results were then analyzed statistically using student t-test and analysis of variance (ANOVA).

RESULTS

Genetic damage in both of groups was assessed using micronuclei assay in buccal epithelial cells. The results are summarized in Tables 1-5. Table 1 revealed the mean values of demographic characteristics, that is, age, diet and alcohol consumption in control and exposed groups. Years of pesticide exposure and use of protective measures by pesticide distributors are also given.

Table1: Mean values of demographic characteristics of the control and exposed group

Characters	*Control*	*Exposed*
Age (Mean± S.D)	41.34±1.55	41.02±1.64
Diet		
Vegetarians	31 (62%)	12(24%)
Non-vegetarian	19 (38%)	38 (76%)
Alcoholic	06 (12%)	33 (66%)
Non- alcoholic	44 (88%)	17 (34%)
Years of pesticide exposure (Mean± S.D)		16.26±1.57
Protective measures taken		02 (4.0%)
No protective measures taken		48 (96%)

Comparison of micronuclei assay end points among controls and pesticide exposed population is summarized in Table 2. It was evaluated that mean frequency of miconucleated cells (MN) and binucleated cells (BN) was significantly higher in pesticide exposed population as compared to controls, values of t being 7.61 and 4.08 for MN and BN respectively.

Table2: Showing frequency of Micronucleated (MN) and binucleated buccal epithelial cells in controls and pesticide distributors

Study groups	*Frequency of MN cells/1000 cells (%,Mean± S.E)*	*Frequency of BN cells (%,Mean± S.E)*
Control (n=50)	1.08±0.19	1.24±0.23
t-value	**7.61****	**4.08****
Exposed (n=50)	3.78±0.29	3.08±0.38

** =Significant at 1%

To evaluate the effect of duration of pesticide exposure the studied population was divided into four groups according to years of exposure, that is, 01-10, 11-20, 21-30 and 31-40. Figure 1 showed increasing trend in frequency of MN and BN cells as revealed from one way ANOVA, values of F being 13.83 and 6.29 respectively for MN and BN. To assess the effect of age on genetic damage population was divided into four age groups, that is, 20-30, 31-40, 41-50 and 51-60. One way ANOVA was applied to assess the effect of age on the MN assay end points. It was revealed that significant positive association between genetic damage age was found for control (for MN $F=5.44$, $p\leq0.005$ and for BN $F=5.49$, $p\leq0.005$). Similarly significant association was also observed in exposed group (for MN $F=6.47$, $p\leq0.005$ and for BN $F=5.06$, $p\leq0.005$) (Table 3). The effect of diet on the DNA

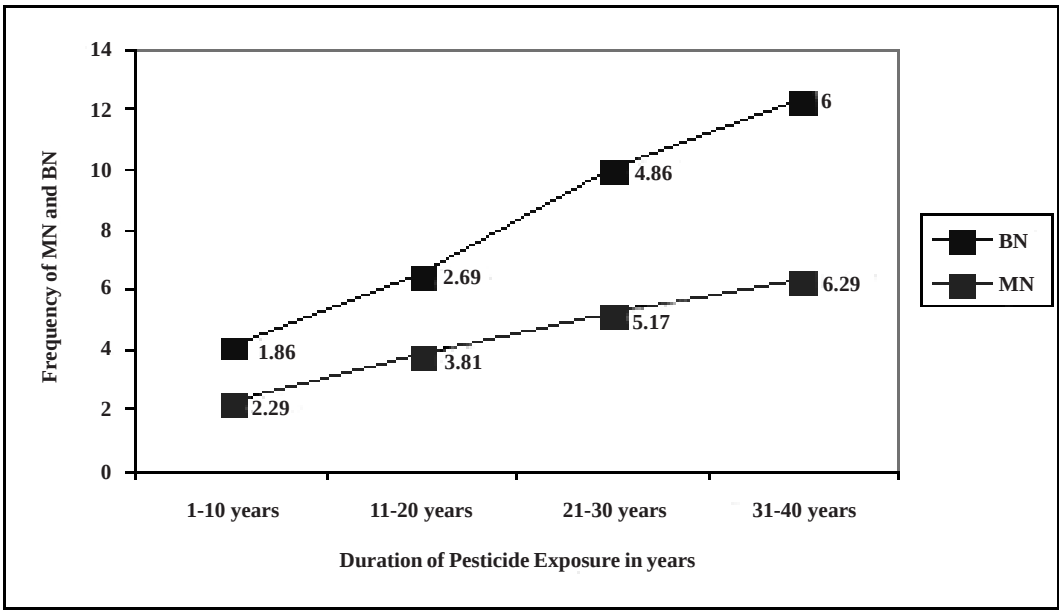

Fig. 1. Effect of duration of exposure on frequency of MN and BN cells in buccal epithelial cells in pesticide distributors

damage is presented in Table 4. It was observed that the values of frequency of micronucleated (MN) and binu-cleated cells (BN) showed increased values in case of non- vegetarians for both the populations, that is, control and exposed. However statistically non- significant difference was observed for both the parameters except for frequency of MN cells in exposed group where the difference was found to be statistically significant (t=2.04), p<0.005) values being 3.67±0.58 for vegetarians and 3.81±0.35 for non-vegetarians.

Table 4: Mean frequency of MN and BN cells in buccal mucosa of controls and pesticide distributors according to diet

Parameter		*Control*	*Exposed*
Frequency of MN cells/1000 cells (Mean± S.E)	Vegetarians	0.77±0.18 (n=31)	3.67±0.58 (n=12)
	Non-vegetarians	1.58±0.40 (n=19)	3.81±0.35 (n=38)*
Frequency of BN cells/1000 cells (%, Mean± S.E)	Vegetarians	0.93±0.23 (n=31)	2.33±0.50 (n=12)
	Non-vegetarians	1.73±0.48 (n=19)	3.32±0.48 (n=38)

*=Significant at 5%

Table 3: Showing mean frequency of MN and BN cells (%, Mean± S.E) in buccal epithelial cells with respect to the age in controls and pesticide distributors

Study group	*No. of subjects*	*Frequency of MN cells/1000cells (%, Mean±S.E)*	*Frequency of BN cell/1000cells (%, Mean±S.E)*	*f-values*	
				MN	*BN*
Control Age Groups					
(I) 20-35	11	0.33±0.19	0.18±0.12	**5.44***	**5.49***
(II) 36-50	12	0.36±0.24	0.50±0.29		
(III) 51-65	14	1.43±0.52	1.86±0.63		
(IV) 66-85	13	2.00±0.25	2.15±0.27		
Exposed Age Groups					
(I) 20-35	11	2.00±0.34	1.77±0.44		
(II) 36-50	12	3.92±0.30	2.42±0.70	**6.47***	**5.06***
(III) 51-65	14	4.25±0.54	2.67±0.54		
(IV) 66-85	13	4.84±0.70	5.39±0.92		
t-values		**6.97****	**3.73****		

** =Significant at 1%; * = Significant at 5%

The effect of alcohol consumption is revealed in Table 5. It was examined that although, mean values of frequency of micronucleated (MN) and binucleated (BN) cells were higher in alcoholics as compared to non alcoholics for both control and exposed group yet the difference was statistically non- significant.

Table 5: Mean frequency of MN and BN cells in buccal mucosa of control and exposed groups according to alcoholic habit

Parameter		*Control*	*Exposed*
Frequency of MN cells/1000 cells (Mean± S.E)	Alcoholic	1.50±0.61 (n=06)	4.00±0.39 (n=33)
	Non-alcoholic	1.02±0.20 (n=44)	3.53±0.42 (n=17)
Frequency of BN cells/1000 cells (%, Mean± S.E)	Alcoholic	1.50±0.56 (n=06)	3.53±0.42 (n=33)
	Non-alcoholic	1.18±0.25 (n=44)	2.71±0.36 (n=17)

DISCUSSION

Several groups of workers are potentially exposed to pesticides on the basis of their occupation and the possible health effects of these chemicals are constantly being scrutinized. Pesticide distributors are an important link in pesticide distribution chain in India. The distribution of pesticides for agricultural purposes is mainly through the distributors (retail shops/ whole sale shops) and hence these distributors particularly the workers engaged in dealing and handling of mixtures of pesticides in India and many developing countries are more prone to hazardous effects caused by pesticides.

Genetic monitoring of populations exposed to potential carcinogens is an early warning for genetic disease or cancer. It allows identification of risk factors at early stages when control measures could still be implemented (Kassie et al. 2000). Thus the present investigation was carried out to assess the genetic damage in the pesticide distributors (a population that is occupationally exposed to pesticides) of Punjab (Amritsar, Jalandhar and Jandiala). Parameters taken into consideration were frequency of micronucleated (MN) and binucleated (BN) cells in all the cases.

In the present study a significant increase was found in the frequency of micronucleated and binucleated cells among pesticide distributors as compared to control population. A number of studies (both in vivo and in vitro) are in agreement with the present findings in spite of the fact that the selection criterion of different techniques and different kinds of pesticide exposures in different studies (Undeger and Basaran 2002; Grover et al. 2003; Bhalli et al. 2006; Silva et al. 2008).

Many factors have been reported to produce effects on the values of genetic markers viz age, exposure, diet, gender, smoking and season (Moller et al. 2000). For the evaluation of the effect of duration of pesticide exposure entire population was divided into four subgroups ranging from 1-40. A number of earlier studies have reported a relationship between genetic damage and duration of exposure (Zeljezic and Garaj-Vrhovac 2001; Bolognesi et al. 2002; Grover et al. 2003; Bhalli et al. 2006) that are in agreement with our findings. Also a temporary increase in DNA damage and MN has been reported during intense spraying season in low exposure period (Antonucci and de Syllos Colus 2000; Bolognessi et al. 2002).

For the assessment of effect of age of pesticide distributors and control population both the study populations were divided into four groups. Age showed significant effect on both the parameters. Few studies have reported a significant association between genetic damage with increase in age (Chadha and Yadav 2011; Gentile et al. 2012).

An attempt was also made to analyse the genetic damage with the dietary habits. Diet did not show positive correlation with genetic damage in both the parameters. Similar results were also reported by Dhawan et al. (2001), Pastor et al. (2002) and Chadha et al. (2013). However, the mean values of non- vegetarians were higher than vegetarians for both the study groups. It can be assumed that diet of workers which is made up of large amounts of natural food and vegetables may reduce the clastogenic effects of toxicants. There are evidences that certain vegetables contain anticlastogenic agents such as galangin (Hoyos et al. 1996). It is found that vitamin C, vitamin E, carotenoids and flavonoids are free radical scavengers (Duthei and McMillian 1997). When the effect of alcohol was examined for genotoxic damage no positive association was found between alcoholics and non alcoholics of exposed population for both the parameters Similar findings were studied by (Sailja et al. 2006; McCauely et al. 2008). On the contrary some studies have found rela-

tionship between alcohol consumption and alterations in the normal oral mucosa as well as increase in MN in buccal epithelial cells (Kassie et al. 2000).

The utilization of individual protective measures by the exposed workers also influences the toxic effects. The MN frequency was found to be higher in workers those who did not use gloves during application as compared to those that use any kind of protection (Bull et al. 2006; Sailja et al. 2006; Costa et al. 2007). Similarly, an elevation in SCE (Padmavathi et al. 2000) and MN (Bolognesi et al. 2002) was seen in pesticide exposed persons who did not use protective measures. However, despite using protective equipment and procedures (like masks, protective clothing and shower before and after application) Silva et al. (2008) noticed an increase in MN frequency among pesticide applicators with no difference between those who used complete protective equipment and those who did not. For present study nothing conclusive can be said as only two out of fifty workers used a sort of mask.

Conclusively, the positive findings of increased genetic damage in the buccal mucosal epithelial cells of pesticide distributors in the present work indicates the potential genetic hazards posed by excessive use of pesticides in Amritsar, Jalandhar and Jandiala. So the use of protective measures and other safety regulations should be emphasised among the pesticide distributors to prevent further exposure. Further more it is very important that surveillance be maintained for the high risk group which had an increased genetic damage.

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