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Frequencies of SCEs in Peripheral Blood Lymphocytes of Pesticide Workers

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In recent years there has been increased awareness of problems of human exposure to potentially toxic chemicals in the work place. Although the work environment has improved by setting certain control limits, it may still prove hazardous over the long term. The World Health Organization has been recommending increased efforts to generate data on individuals with different occupational exposures.

Genetic damage at the chromosome level is an alteration either in chromosome number or in chromosome structure. Such alterations can be observed as chromosomal aberrations and sister-chromatid exchanges (SCEs) and can be accurately assessed in somatic cells. These are currently the most widely used endpoints for evaluating somatic mutations. Hence it is appropriate to use these parameters to assess cytogenetic damage in industrial workers.

Previously studies have been carried out to evaluate cytogenetic effects in pharmaceutical workers (Pushpavathi et al. 1986), cement factory workers (Fatima et al. 1995), leather factory workers (Berrin and Unal 1994), nuclear fuel workers (Prabhavathi et al. 1995), petroleum processing workers (Simenova et al. 1989), chemical factory workers (Ziqiang and Lianzhen 1990), anesthetists (Natarajan and Santhiya 1990; Padmavathi et al. 1995), zinc smelting workers (Bauchinger et al. 1976), electroplating workers (Stella et al. 1982) and pesticide sprayers (Cheng et al. 1988; Rupa et al. 1989a,b,c; Arroyo et al. 1992; Kourakis et al. 1992).

Pesticide industry workers form a high risk group as they are occupationally exposed not only to the pesticides which they manufacture but also to a wide range of toxic chemicals that are used as raw materials. The pesticide industry commonly use chemicals like formaldehyde, chlorine, hydrogenchloride, phosphorus pentasulphide, dichloroethane, acrylonitrile and organic solvents like ethylalcohol, benzene, toluene and carbontetrachloride. Some of these chemicals, especially the organic solvents have been identified as clastogenic agents (William et al. 1990; Slozina et al. 1990).

As reports on SCEs in pesticide industrial workers are scanty, we attempted to evaluate cytogenetic damage in pesticide industrial workers by examining frequencies of SCEs in peripheral blood lymphocytes.

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MATERIALS AND METHODS

A total of 135 workers from an organophosphorus pesticide industry in Hyderabad, India with an age range of 22-47 years and with duration of exposure ranging from 1 to 24 years, formed our study group. There were 83 non-smokers and 52 smokers in the exposed group. A total of 111 individuals who were between 24 to 49 years old and of the same socio-economic status as the exposed study group formed the control group. The control group consisted of 65 non-smokers and 46 smokers. A standard questionnaire was used to collect relevant information on smoking and drinking habits, medication and exposure to physical and chemical mutagens. Workers used few protective measures and they worked 8 hours per day for 6 days a week throughout the year.

Intravenous blood was collected and 0.5 ml of whole blood from each sample was cultured in RPMI 1640 medium supplemented with 25% human AB serum 0.5% phytohaemagglutinin-P and 0.25% antibiotic. Bromodeoxyuridine (3 µg/ml Brdu) was added to cultures at the time of initiation. The culture vials were wrapped in black paper and incubated at 37°C for 72 hours. Colchicine solution (0.3 µg/ml) was added 2 hours before harvesting of cultures to arrest cell division at metaphase stage. The cultures were harvested and slides were prepared using the standard method of Moorhead et al. (1960). All slides were coded and stained after 3 days using the fluorescence plus giemsa technique of Perry and Wolff (1974). For each sample, 50 well spread metaphases with good differential staining were scored for SCEs. The results were statistically analysed using one factor ANOVA.

RESULTS AND DISCUSSION

The mean number of SCEs in the exposed group of non-smokers (7.61 ± 2.288) was significantly greater than in the control group (4.12 ± 0.801). The exposed group when categorised into ≤10 and ≥ 11 year groups according to their period of service showed frequencies of SCEs of 6.91 ± 1.952 and 8.60 ± 2.387 respectively. A significant increase in SCE / Cell was observed at all exposure intervals when compared to the control group ($p < 0.001$) and the difference in SCE / Cell between exposure intervals was also found to be significant ($p < 0.05$).

The mean SCE / Cell in smoker control group was 5.92 ± 2.611 compared to 9.68 ± 3.026 in smoker exposed group ($p < 0.001$, Table). A statistically significant difference was also observed between the non-smoking controls (4.12 ± 0.801) and smoking controls (5.92 ± 2.611). Analysis of SCEs in pesticide workers showed a significant increase with years of service; 8.97 ± 2.637 and 10.64 ± 3.310 with ≤ 10 and ≥ 11 years of service respectively. The increase in the mean SCEs was significant at all year intervals when compared with the control group of smokers. The difference in mean SCE / Cell inbetween the year intervals was also found to be statistically significant ($p < 0.05$).

Table-1 : Frequencies of sister - chromatid exchanges (SCEs) in pesticide industry workers

Group / duration of exposure in years	Non-smokers SCE / Cell Mean ± SD	Smokers SCE / Cell Mean ± SD
Control group	4.12^{a} ± 0.801 (65)	5.92^{a} ± 2.611 (46)
Exposed group		
≤ 10 years	6.91^{b} ± 1.952 (48)	8.97^{b} ± 2.637 (30)
≥ 11 years	8.60^{c} ± 2.387 (34)	10.64^{c} ± 3.310 (22)
Total	7.61 ± 2.288 (82)	9.68 ± 3.026 (52)
F ratio	87.99	24.45
Level of significance	$p < 0.001$	$p < 0.001$

Note : 1. Values in the parentheses indicate the sample size
2. Variation in superscripts between groups for given smoking habits indicates significance of difference ($p<0.05$)

Sister-chromatid exchanges are sensitive indicators of chromosomal damage caused by a variety of physical and chemical agents (Popescu et al. 1977; Wolff 1977). Several studies have demonstrated that the frequency of SCEs increase when cells, animals or humans are exposed to known mutagens or carcinogens (Latt et al. 1981; Agamohammadi et al. 1988).

The results of the present study showed a high frequency of SCEs in pesticide industry workers. This is in accordance with previous studies on agricultural workers exposed to pesticides. Dulout et al. (1985) reported SCE frequency of 5.47 ± 1.03 in symptomatic and 6.45 ± 1.19 in asymptomatic floriculturists occupationally exposed to organochlorine, organophosphorus and carbamate pesticides. A significant elevation in SCEs was reported in workers exposed to dichlorvos (Cheng et al. 1988) Rupa et al. (1988) reported SCE frequencies of 10.94, 10.96, 1188, 11.16, 12.36, 12.58 in 5-10, 11-15, 16-20, 21-25, 26-30 and 32 year group respectively in vegetable garden workers. In another study Rupa et al. (1989b) reported significant increase in SCE frequencies with years of exposure in smoking pesticide sprayers; 7.85, 9.63, 10.50, 10.68 and 11.18 with 1-5, 6-10, 11-15, 16-20 and 21-25 years of exposure respectively. It was also reported that the SCE / Cell at all the exposure intervals was significantly higher when compared to the non-smoking (5.42) and smoking controls (7.60). The present study also

showed a higher frequency of SCEs in smoking workers when compared to non-smoking workers, which is in accordance with the study of Kourakis et al. (1996) who showed significant increases in a Spanish group of agricultural workers and also increased frequency of SCEs in the smoking group when compared to the non-smoking group. Scarpato et al. (1996) reported 17% increase in the frequency of SCEs in the smoking group compared to the non-smoking group of Italian floriculturists.

Studies carried out in different test systems have reported the mutagenic effects of several organophosphorus pesticides (Garry et al. 1990; Nurzhanova et al. 1994). Epidemiological studies on agricultural workers have reported incidence of cancers like leukemia (Ciconne et al. 1993; Faustini et al. 1993), bladder cancer (Vie1 and Challier 1995), non-Hodgkin's lymphoma (Sbrana and Musio 1995), renal cell cancer (Schlehofer et al. 1995), brain tumors (Heineman et al. 1995), and gastric and skin cancers (Faustini et al. 1993). Several earlier studies have established the carcinogenic effects of organophosphorus pesticides to lower and higher organisms (Millikan et al. 1995; Sbrana and Musio 1995). Collectively these mutagenic and carcinogenic studies suggest an elevated risk in individuals who are occupationally exposed to pesticides.

In the present study, the analysis of SCEs has shown clear evidence for the clastogenic effect of occupational exposure to pesticides and chemicals in pesticide industry workers. Given that workers are frequently exposed to a variety of organophosphorus pesticides and toxic chemicals, it is difficult to attribute damage to any particular agent.

The clastogenic effect may be due to cumulative effects of all pesticides and chemicals. The occupational exposure to pesticides and chemicals in pesticide industry workers seems to be potentially hazardous. Hence the conditions at the work place should be improved to minimise exposure to pesticides and chemicals. There is a need to educate workers about the potential hazard of occupational exposure and importance of using protective measures.

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