RESEARCH COMMUNICATION

Genotoxic Effects of Textile Printing Dye Exposed Workers in India Detected by Micronucleus Assay

Sudha Sellappa[1*], Shibily Prathyumnan[1], Shyn Joseph[1], Kripa S Keyan[1], Vellingiri Balachandar[2]

Abstract

The textile printing industry in South India employs a great number of workers that may possibly be exposed to toxic compounds. In the present study, subjects from textile printing units were investigated for the presence of genetic damage in their peripheral blood lymphocytes using micronucleus assay. Proliferation was also investigated using a nuclear division index. It was found that the micronucleus frequency was considerably greater in exposed subjects than in non exposed control subjects, but division was not increased in a statistically significant way. For the time being, this investigation should be considered as a preliminary study in which the influence of potential confounders could be adequately assessed. However, our results are non-ambiguous, indicating a potential health risk in these workers.

Keywords: Genetic damage - occupational exposure - textile printing dyes - peripheral blood lymphocytes

Asian Pacific J Cancer Prev, **11**, 919-922

Introduction

During the last few years, genotoxicity biomarkers have received extensive attention as tools for detecting human genotoxic exposure and effects, particularly in health observation programs dealing with occupational exposure. People are exposed to a large number of physical or chemical agents which can cause a variety of health hazards and human cancers are known to arise as a direct consequence of environmental exposure to mutagenic and carcinogenic agents, mainly through diet, habit and occupation (Moutchen, 1985).

Since exposure to hazardous agents can be identified, actions to decrease their presence in the environment or to protect the population against them can reduce the consequences on community health. One way to study the effects on an exposed population is to conduct monitoring studies, using pertinent biological parameters with a short-term manifestation, such as cytogenetic analysis, by which damage to DNA resulting from exposure can be identified. The obtained information can be used as an early warning about the potential risk of health problems developing in the long run (Au, 1991).

The majority of human populations are exposed to a variety of environmental and/or occupational toxicants. The textile industry is one such source that grew out of the industrial revolution in the 18th century as mass production of clothing became a mainstream industry. Workers of textile industry are mainly exposed to a variety of toxic dyes, bleaching agents, salts, acids, alkalis and heavy metals like cadmium, copper, zinc, chromium, iron etc and possibly carcinogenic compounds such as dyes, organic solvents and fixatives throughout the printing process (IARC, 1997).

Genotoxicological biomonitoring in human population is a useful tool to estimate the genetic risk from an integrated exposure to complex mixture of chemicals. The use of biomarkers associated with these events provides useful tools for the early detection of disease related changes (Kyrtopoulos et al., 2006). The use of the micronuclei (MN) test to detect and quantify the genotoxic risk is well established in several systems, either in vitro or in vivo. MN frequencies in cultured peripheral blood lymphocytes have been used as biomarkers of chromosome damage for several years. MN are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosome fragments or intact whole chromosomes lagging behind at the anaphase stage of cell division. Their presence in cells reflects structural and/or numerical chromosomal aberrations arising during mitosis (Pal et al., 1980; Maluf et al., 2000).

Considering these, we have analyzed the MN frequency in peripheral blood lymphocytes of textile printing workers who are occupationally exposed to a mixture of dyes and chemicals for a long period in this study.

*[1]Department of Biotechnology, Karpagam University, [2]Unit of Human Genetics, Department of Zoology, Bharathiar University, Coimbatore, Tamilnadu, India. *For correspondence : sudhasellappa@yahoo.co.in*

Materials and Methods

Selection of subjects and collection of specimens

A total of 45 individuals (25 experimentals exposed to synthetic textile-printing dyes and 20 controls) were examined in the study. All of them were employed in textile industries located in Coimbatore City, South India. The workers had varying durations of exposure (2-16 years) and they were in the age group of 21-48 years. The experimental group was further branched as smokers (Pelclova et al., 1990), non-smokers (Bolognesi et al., 2002), alcoholics (Pastor et al., 2001) and non-alcoholics (Burgaz, 1995). The control group composed of people working in the textile garments, in Coimbatore city with no history of occupational exposure to textile dyes, but belonged to the same age group and socio-economic status as the workers. The control group was also subdivided into smokers (Pelclova et al., 1990), non-smokers (Pelclova et al., 1990), alcoholics (Di Giorgio et al., 1994) and non-alcoholics (Pal and Brijmohan, 1980). The selection criteria for the subjects were based on a questionnaire that covered standard demographic questions (age, genetic disorders, number of X-ray diagnoses, vaccinations, medication, smoking, alcohol, etc.) and occupational questions (years and nature of exposure). We ensured that the workers and the controls did not markedly differ from each other except for occupational exposure. We also ensured that all the subjects had not been taking any medicines nor had they been exposed to any kind of radiation for 12 months before sampling. The subjects who smoked >5 cigarettes/day at least for 1 year were considered as smokers and those who consumed >120gm of alcohol/day were considered as alcoholics in both groups. All subjects were informed of the objective of the study and gave their consent. The present study was conducted, according to the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens (Carrano, 1988). The institutional ethical committee approved the research procedures used in this study.

Venous blood (3ml) was collected from all subjects using heparinized syringes. The samples were transported on ice to the laboratory and were processed. Micronucleus assay was performed using the collected blood samples.

Laboratory analysis

Cytokinesis-block micronucleus (CBMN) assays were conducted according to the methods described by Fenech and Morley (1985) and Lou et al. (2004). Leukocyte cultures were set up by adding 0.5 ml whole blood to 4.5 ml RPMI 1640 medium supplemented with 15% heat-inactivated fetal calf serum, 1% antibiotics (penicillin and streptomycin) and L-glutamine (all obtained from Gibco). Leukocytes were stimulated by 1% phytohaemagglutinin (PHA; Gibco) and incubated for 72 h at 37°C. A final concentration of 6 μg/ ml cytochalasin B (Sigma) was added to the cultures 44 h later to arrest cytokinesis (Singh et al., 1988). At 72 h of incubation, the cultures were harvested by centrifugation at 1000 rpm for 8 min and treated with a hypotonic solution (2-3 min in 0.075 M KCl at 4°C). Cells were centrifuged thereafter and a 3:1 (v/v) methanol: acetic acid solution was gently added. This fixation step was repeated twice and the resulting cells were resuspended in a small volume of fixative solution and dropped onto clean slides. Finally the slides were stained with Acridine orange (Merck) analyzed under a fluorescent microscope and scored. Number of micronucleated cells (MNC) and number of micronuclei (MN) per 1,000 binucleated lymphocytes served as the indicators. Meanwhile, when 400 lymphocytes were randomly scored, nuclear division index (NDI) was calculated according to the following formula (Lou et al., 2004): NDI=(1N+2×2N+4× >2N)/400 cells (1N= the number of cells with one nucleus; 2N= the number of cells with two nuclei; >2N= the number of cells with > two nuclei).

Statistical analysis

The results of textile printing industry workers were compared with those obtained from non exposed matched controls. Similarly the results of textile printing industry workers having smoking habits were compared with matched non exposed control smokers, correspondingly for the alcoholics and non-alcoholics as well. The independent variable (sex) was classified as qualitative (nonparametric). The role of nonparametric factors was analyzed by the Mann-Whitney test, while the Student's 't' test was used for age and time comparisons. The number of micronucleated cells and the number of micronucleus in each cell were compared with the Mann-Whitney test.

Results

The demographic characteristics of the study subjects who took part in this study are presented in Table 1. The individuals were classified according to their age, sex, smoking habits, alcohol consumption and duration of work.

The results of CBMN assay are seen in Tables 2 and 3. The ranges of MN in 25 workers and 20 controls were 0-39 and 0-7, respectively, while the average MN of workers was 10±7.4 which was significantly higher than that (2.65±1.84) of controls (P<0.01). The ranges of MNCs of workers and controls were 0-32 and 0-5, respectively. The average MNCs of workers and controls were 9.04±6.4 and 2.4±1.4, respectively. The difference between the average MNC of workers to that of controls was remarkably significant (P<0.01). The distribution of

Table 1. General Characteristics of the Study Group and Controls

Characteristics	Exposed(N=25)	Controls(N=20)
Mean age (years)	27.54 ± 5.36	28.30 ± 5.38
Age range (years)	21- 48	19 - 42
Males	14 (56%)	10 (50%)
Females	11 (44%)	10 (50%)
Smokers	10 (40%)	10 (50%)
Non-smokers	15 (60%)	10 (50%)
Alcoholics	14 (56%)	12 (60%)
Non-alcoholics	11 (44%)	8 (40%)
Mean time working (years)	5.35 ± 2.54	-
Range of time working (years)	2-16	-

Table 2. The Results of CBMN Assays in Controls

Controls	Sex	S	A	No. of cells carrying micronuclei			Total no of MNC	Total no of MN	NDI (%)
				1MN	2MN	3MN			
C01	F	N	N	2	0	0	2	2	1.21
C02	M	Y	Y	3	0	0	3	3	1.41
C03	M	Y	Y	3	1	0	4	5	1.24
C04	M	Y	N	2	0	0	2	2	1.56
C05	F	N	N	1	0	0	1	1	1.41
C06	M	Y	Y	3	0	1	4	6	1.61
C07	F	N	N	2	0	0	2	2	1.71
C08	M	Y	Y	3	0	0	3	3	1.29
C09	F	N	N	1	0	0	1	1	1.42
C10	M	Y	Y	3	0	0	3	3	1.75
C11	F	N	N	1	0	0	1	1	1.22
C12	M	Y	Y	4	0	0	4	4	1.24
C13	F	N	N	1	0	0	1	1	1.51
C14	F	N	N	1	0	0	1	1	1.10
C15	M	Y	Y	5	0	0	5	5	1.10
C16	F	N	N	2	0	0	2	2	1.24
C17	M	Y	Y	3	2	0	5	7	1.61
C18	F	N	N	1	0	0	1	1	1.34
C19	F	N	N	1	0	0	1	1	1.21
C20	M	Y	N	2	0	0	2	2	1.24
Mean±SD				2.2 ± 1.15	0.15 ± 0.49	0.05 ± 0.22	2.4 ± 1.4	2.65 ± 1.84	1.37 ± 0.20

MNC, micronucleated cell; MN, micronuclei; NDI, nuclear division index; S, Smokers; A, Alcohol users

Table 3. The Results of CBMN Assays in Workers

Group				No. of cells carrying micronuclei			Total no of MNC	Total no of MN	NDI (%)
				1MN	2MN	3MN			
Workers	Sex	S	A						
W01	M	N	Y	6	2	0	8	10	1.14
W02	F	N	N	9	0	0	9	9	1.21
W03	M	Y	Y	25	7	0	32	39	1.04
W04	M	Y	Y	11	0	0	11	11	1.94
W05	M	N	Y	7	1	0	8	9	1.03
W06	F	N	N	1	0	1	2	4	1.10
W07	M	N	Y	10	0	0	10	10	1.31
W08	F	N	N	4	0	0	4	4	1.52
W09	F	N	N	0	1	0	1	2	1.21
W10	F	N	Y	6	2	0	8	10	1.02
W11	M	Y	Y	15	0	0	15	15	1.21
W12	M	Y	Y	11	0	0	11	11	1.52
W13	M	Y	Y	14	1	0	15	16	1.62
W14	F	N	N	3	0	0	3	3	1.30
W15	M	Y	Y	7	1	1	9	12	1.02
W16	F	N	N	1	0	0	1	1	1.06
W17	M	Y	Y	9	1	0	10	11	1.11
W18	F	N	N	4	0	0	4	4	1.18
W19	F	N	N	7	0	0	7	7	1.14
W20	M	Y	Y	14	0	0	14	14	1.52
W21	M	Y	Y	11	1	0	12	13	1.92
W22	M	N	N	9	0	0	9	9	1.21
W23	F	N	N	5	0	0	5	5	1.23
W24	F	N	N	3	0	1	4	6	1.14
W25	M	Y	Y	13	1	0	14	15	1.26
Mean±SD				8.2 ± 5.52	0.72 ± 1.46	0.12 ± 0.33	9.04 ± 6.4[a]	10.00 ± 7.4[a]	1.28 ± 0.26

MNC, micronucleated cell; MN, micronuclei; NDI, nuclear division index; S, Smokers; A, Alcohol users; [a] $P < 0.01$, workers compared with controls.

micronuclei per cell and the NDIs of workers and controls are listed in Tables 2 and 3. The ranges of NDIs of workers and controls were 1.02-1.94 and 1.10-1.71, respectively. The average NDIs (1.28±0.26) of workers was lower than that (1.37±0.20) of controls, but the difference between two groups for NDI was not significant ($P > 0.05$).

Discussion

India is one of the major centers of textile industry which consumes a large amount and variety of dyes. In the dyeing, printing and finishing processes, workers frequently have multiple exposures, which can vary with

time and process (Luce et al., 1993). Textile industry poses threat of various types of occupational diseases (Pal et al., 1910). Micronucleus assays have emerged as one of the preferred methods for assessing chromosome damage applied to various cell types for population monitoring. In its current basic form the CBMN assay can provide, using simple morphological criteria, the following measures of genotoxicity and cytotoxicity: chromosome breakage, chromosome loss, chromosome rearrangement (nucleoplasmic bridges), cell division inhibition, necrosis and apoptosis (Fenech, 2000).

The significant increase in the frequency of MN in leukocytes of textile printing workers could be the result of continuous absorption of chemicals which contain genotoxic substances. The mean NDI of workers was lower than that of controls but with no significant difference. The exposed group had an increased MNC frequency, as compared to controls ($p<0.01$) and a slight difference was found between the sub groups of exposed individuals. This result is in conformity with the findings of Ragunathan et al., (2007), who investigated chromosome alterations in textile dyeing workers. Increase in the chromosomal aberration was noted in workers exposed to benzidine based dyes (Mirkova et al., 1990). Similarly chromosome analysis of peripheral lymphocytes of workers exposed to rotogravure printing dyes showed an increased incidence of aberrant cells and chromatid breaks (Pelclova et al., 1990). In biomonitoring studies of populations occupationally exposed to genotoxic agents, the smoking habit influence on MN frequency is controversial. Some studies have provided evidence of an association (Di Giorgio et al., 1994; Burgaz et al., 1995), but most of the studies have not (Pastor et al., 2001; Bolognesi et al., 2002; Bonassi et al., 2003).

In the present study, we observed a small and non-significant increase in MN among exposed individuals with smoking habit. Our results suggest that smoking habits are not related to the levels of MN in both the control and exposed group. Similarly Maluf and Erdtmann (2000) have also reported no differences in the number of MNC in cultured peripheral leucocytes between smokers and non-smokers. There appear to be contradictory results regarding the influence of smoking on nuclear alterations.

The textile printing workers had an increased frequency of MN, due to the genotoxic action of the substances to which they are exposed in their work. The leucocytes express the action of environmental agents with genotoxic potential. Consequently, MN of this type of tissue provides a good evaluation of the degree of occupational exposure. The study and the standardization of tests for the evaluation of biological damage is necessary to guarantee environmental quality and occupational health, as well as to orient workers to help reduce genetic damage and the severe health risk.

Acknowledgements

The authors are grateful to the authorities of Karpagam University and Dr. K. Sasikala, Unit of Human Genetics, Department of Zoology, Bharathiar University, for their open handed support and encouragement.

References

Au CC (1991). Cytogenetic assays in monitoring human exposure and prediction of risk. *Environmental Mutagenesis Carcinogenesis Teratogenesis*, **23**, 236-45.

Bonassi S, Neri M, Lando C, et al (2003). Effect of smoking habit on the frequency of micronuclei in human lymphocytes: Results from the human micronucleus project. *Mutat Res*, **543**, 155-6.

Bolognesi C, Perrone E, Landini E (2002). Micronucleus monitoring of a floriculturist population from Western Liguria, Italy. *Mutagenesis*, **17**, 391-7.

Burgaz S, Iscan A, Buyukbingol ZK, et al (1995). Evaluation of micronuclei in exfoliated urothelial cells and urinary thioether excretion of smokers. *Mutat Res*, **335**, 163-9.

Carrano AV (1988). Considerations for population monitoring using cytogenetic techniques. ICPEMC-International commission for protection against environmental mutagens and carcinogens. Publication n. 14. *Mut Res*, **204**, 379-406.

Di Giorgio C, De Méo MP, Laget M, et al (1994). The micronucleus assay in human lymphocytes: screening for inter-individual variability and application to biomonitoring. *Carcinogenesis*, **15**, 313-7.

Fenech M, Morley AA (1985). Measurement of micronuclei in human lymphocytes. *Mutation Res*, **148**, 29-36.

Fenech M (2000). The in vitro micronucleus technique. *Mutat Res*, **455**, 81-95.

IARC (1997). Monographs on the evaluation of carcinogenic risk to humans. Lyon. *IARC*, **69**, 545-51.

Kyrtopoulos SA, Sarrif A, Elliott BM, et al (2006). Biomarkers and molecular epidemiology-present state and future trends: concluding remarks. *Mutat Res*, **600**, 77-8.

Luce D, Gérin M, Leclerc A, et al (1993). Sinonasal cancer and occupational exposure to formaldehyde and other substances. *Int J Cancer*, **1**, 224-31.

Lou JL, He JL, Jin LF, et al (2004). Measuring the genetic damage in cancer patients during radiotherapy with three genetic end-points. *Mutagenesis*, **19**, 457-64.

Maluf SC, Erdtmann B (2000). Evaluation of occupational risk in a Brazilian hospital. *Genet Mol Biol*, **23**, 485-8.

Mirkova ET, Lalchev SG (1990). The genetic toxicity of the human carcinogens benzidine and benzidine based dyes: chromosomal analysis in exposed workers. *Prog Clin Biol Res*, **340**, 397-405.

Moutchen J (1985). Introduction to genetic toxicology. Wiley New York; 0471901431:184.

Pal PB, Brijmohan (1980). Management of occupational environment in textileindustry. *Indian J Environ Prot*, **10**, 767-72.

Pastor S, Gutiérrez S, Creus A, et al (2001). Cytogenetic analysis of Greek farmers using the micronucleus assay in peripheral lymphocytes and buccal cells. *Mutagenesis*, **16**, 539-45.

Pelclova D, Rossner P, Pickova J (1990). Chromosome aberrations in rotogravure printing plant Corkers. *Mut Res*, **245**, 299-303.

Ragunathan I, Palanikumar L, Panneerselvam N (2007). Cytogenetic studies on the peripheral lymphocytes of occupationally exposed textile dye industry workers. Facta universitatis series. *medicine and biology*, **14**, 43-6.

Singh NP, McCoy MT, Tice RR, et al (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res*, **175**, 184-91