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Elevated frequencies of micronuclei and other nuclear abnormalities in buccal epithelial cells of spray painters in South India

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Abstract

Human biomonitoring using the micronucleus (MN) assay on exfoliated buccal cells is an invasive approach for the assessment of genetic damage in exposed populations. To determine the genotoxic effects of spray painters, MN assay was carried out in exfoliated buccal epithelial cells of 94 spray painters and 80 controls. From each individual, 2,000 exfoliated buccal cells were analyzed. The individuals used in the study were grouped based on their smoking and alcohol habits. There was a significantly elevated occurrence of micronucleated cells in the exposed workers than in controls**.** Spray painters showed a significant increase in MN when compared to controls with respect to their smoking and drinking habits, age and years of exposure. The association of cytogenetic damage with spray painting exposure gives important information to risk assessment process and concentration should also be paid towards the implementation of personal protective measures.

Key-Words: Micronucleus, Occupational exposure, Spray painters, Exfoliated cells, Genotoxicity

Introduction

Populations of industrial areas are intensely exposed to chemical substances that can cause mutations, cancer, and congenital defects.[1] Humans are diverse in their responses to exogenous exposures because of variability, the rate of metabolism, DNA repair processes and other factors.[2] Occupational health deals with all aspects of health and safety in the workplace and has a strong focus on primary prevention of hazards. The health of the workers has several determinants, including risk factors at the workplace leading to cancer. Painters are exposed to complex mixtures of organic solvents (aliphatic, aromatic, and chlorinated), metals (lead, chromium, cadmium) and many other compounds with potential mutagenic properties.[3]

Occupational agents can induce several types of cancer, such as urinary tract, skin, larynx, and pancreas cancers, and leukemias.[4] Car spray painters are exposed to the action of a great number of chemicals, such as solvents whose foundation is ketone, aliphatic and aromatic compounds and esters, organic and inorganic pigments and several types of resins.

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Natural Increased frequencies of micronuclei in lymphocytes and in buccal epithelial cells were observed among paint industry workers, which were attributed to working conditions, mostly to the organic solvents present in the working areas.[5] Similar considerations were made by Silva and Santos-Mello, [6] who observed increased proportions of aneuploid lymphocytes and chromosome deletions in car painters and by Fuchs et al., [7] who reported a transient increase in DNA strand breaks in car spray painters.

One way to study the effects on an exposed population is to conduct biomonitoring studies, using pertinent biological parameters with a short term manifestation, such as cytogenetic analysis, by which damages to the DNA or to the chromosomes resulting from exposure can be identified. Micronucleus (MN) assay for exfoliated cells have been used to evaluate the genotoxic effects produced by low doses of carcinogenic substances or carcinogenic mixtures, to which human populations are exposed.[8]

Knowledge of human health risks related to environmental exposure to hazardous chemical agents is a current concern[9]. In South India automotive workshops are located on streets and workers at the workshops, have a higher opportunity for exposure. To add further knowledge to the genetic risk on an exposed population, we applied the MN and other

nuclear abnormalities (NA) as a biomarker. Our objective was to evaluate the cytogenetic damage in exfoliated buccal cells obtained from spray painters and control subjects and to establish the relationship of the MN frequency with non-occupational factors, such as the smoking and drinking habits.

Material and Methods

Subjects

The study population composed of 94 male spray painters and 80 unexposed controls. The exposed group included 28 smokers and 22 non-smokers, 26 alcoholics and 18 non-alcoholics, from 21 automotive workshops located in the rural area of Coimbatore City, South India. The respective control groups were matched for age and sex (24 smokers and 18 non-smokers, 21 alcoholics and 17 non-alcoholics) and had no occupational exposition to toxic agents. At the time of sample collection the subjects signed a term of informed consent. All subjects were selected based on questionnaire which included items about age, occupational exposure, smoking habit, use of drugs, such as alcohol, virus illnesses, recent vaccinations, and radiological exams. All the individuals who agreed to participate in the study were healthy, and they answered a detailed questionnaire according to the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens.[10] For the exposed group, a further questionnaire was completed to evaluate the use of protective measure. These workers were engaged in work for more than 8 hrs per day with a minimum 5 yrs of exposure duration. None of these study groups showed significant differences with regard to lifestyle and personal factors. The study procedures used in the present study were approved by the Institutional ethics committee.

Cell sampling

Before sampling, each subject rinsed the mouth thoroughly with tap water. Buccal cells (BCs) were collected from consented volunteers at the end of the work shift according to the criteria established by Tolbert and his co-workers.[11] Prior to BC collection the mouth was rinsed thoroughly with water to remove any unwanted debris. Buccal cell samples were obtained by rubbing the inside of both cheeks using a wooden spatula. The cells were collected in tubes containing 3ml sterile saline.

Micronucleus analysis

Ten micro liters of buccal mucosal cell suspension was smeared on a microscopic slide, dried in air and fixed with cold methanol: acetic acid (3:1) solution in 0.1M phosphate buffer (pH 7.5) for 20 min. Then the slides were stained by Feulgen reaction essentially by the modified procedure of Belien and co-workers.[12] In briefly, hydrolysis in 5N HCl for 10 min at room temperature, washing in distilled water for 5 min, staining with fresh Schiff reagent (Sigma Chem, USA) for 90 min, and washing in tap water for 15 min. The cells were counter stained in Coplin jars containing 1% Fast Green reagent for 2-5 min and rinsed with distilled water. Slides were analyzed under light microscope (Leitz, Germany) with 1000 x magnification. A total of minimum 2000 cells per individual were scored for analysis of micronuclei. The slides were randomized and scored by a single observer. Micronuclei were scored in normal cells. In addition, the frequencies of nuclear anomalies, namely binucleates, broken eggs, karyolysis were recorded. MN and other nuclear abnormalities were classified according to Tolbert and co-workers. MN must satisfy the following conditions: a) consist of nuclear material; b) be completely separated from the parent nucleus; c) be less than 1/3 of the diameter of associated nuclei; d) be smooth, oval- or roundshaped; e) be on the same plane of focus and f) be of the same color, texture and refraction as the main nucleus. Cells with two nuclei were considered to be binucleate. Besides MN, other NA, such as Binucleates (BN), broken eggs' (nuclei that appeared cinched), karyolysis (dissolution of nucleus) were recorded separately.

Statistical analysis

The samples were coded at the time of preparation and scoring. They were decoded before statistical analysis for comparison. The significance of the differences between control and exposed group means were analyzed using Student's *t*-test, whereas Pearson's rank correlation analyses were performed to assess the association between end-points and the independent variables. The MNC, BNC, BEC and KLC distributions of individuals, grouped by each of two-class factors, were compared with the Mann-Whitney test. All the calculations were performed using SPSS 11.01 statistical software (SPSS Inc., Chicago, IL).

Results and Discussion

The characteristics of the subjects used in the study are shown in Table 1. The individuals were classified according to their age, length of occupation, smoking, tobacco chewing, and alcohol drinking habits.

Results on micronuclei frequency and nuclear abnormalities are given in Table 2. Assessment of MN frequencies in exfoliated buccal cells revealed a significant difference ($p < 0.05$) between exposed workers with smoking (12.52 ± 0.21) and controls with smoking habit (3.18 ± 1.36). Smoking also had a marked effect on MN frequency among unexposed control group (3.18 vs. 1.43 between smokers and non-

smokers, respectively). The average MN frequency in the exposed non-smokers was 8.64 ± 1.32, and in the control non-smokers was 1.43 ± 1.08 ($p < 0.05$). The average micronucleus frequencies were 11.59 and 3.96 ± 0.47 between exposed alcoholics and unexposed alcoholics, respectively.

Like MN, A significant difference ($p < 0.05$) in other NA was more prevalent in spray painters compared with that of controls. Among the three NA, karyolysis was predominant in smokers followed by alcoholics. Burgaz et al.[13] reported a significant increase of micronucleated cells ($p < 0.001$) in smokers, as compared to non-smokers. Similarly our study showed synergistic effect between habitual usage and occupational exposure. We observed a significant increase in the frequency of micronuclei formation and nuclear changes in buccal epithelial cells. Individuals of the exposed as well as control groups with smoking habit and alcohol consumption showed an enhanced frequency of micronuclei in comparison to non-smokers and non- alcoholics.

Alcoholic beverages have been described as containing mutagenic substances.[8] Bishop et al.[14] reported that alcohol does not induce mutations in mammal cells in vitro, whereas in vivo it induces a variety of genetic effects, including sister chromatid exchange and the production of micronuclei, where the evidence, however, is limited to certain test systems or tested organisms. According to Dittberner et al.[15] alcohol use can increase the number of micronuclei. The possibility of cytogenetic damage in various occupations exposed to organic solvents has been discussed in several papers.[5,6,16-18] The increasing use and diversity of solvent raises concern about possible risks in occupational exposure. In the present work there is increase in the frequency of induction in the MN in the exposed individual consuming alcohol.

In our study, we used exfoliated buccal cells for the assessment of cytogenetic damage mainly because analysis of buccal epithelium cells provides information about nuclear changes, such as KL (dissolution of the nucleus), the BE effect (broken-egg structures), and BN (two nuclei within a cell). KR and KL are early indicators of apoptosis.[19,20] Higher values of chromosomal aberrations (CAs), sister chromatid exchange (SCE), micronuclei (MN) (in lymphocytes and in oral mucosa cells), and DNA damage detected by the Comet assay in leukocytes are reported for workers exposed to automobile coatings and painters in general.[21,22] Our results on control population also demonstrate that cigarette smoking and alcohol drinking increase in the buccal cells of control population compared to the unexposed non-smoking and alcohol drinking group. The frequency of MN in human exfoliated cells is considered a useful biomarker of genotoxic effects in population exposed to genotoxicants, through direct contact with ingested or inhaled compounds.[23]

Genotoxicity biomarkers have received a considerable interest as tools for detecting human genotoxic exposure and effects, especially in health surveillance programs dealing with chemical carcinogens. Exploration of correlations between biomarkers will contribute to the development of human biomonitoring to genotoxic exposures and will help to select optimal biomarkers for more efficient monitoring of various human exposures.[24]

Conclusion

Occupational and environmental exposures mostly represent mixtures of genotoxic agents, whereas the specificity of biomarker measurements varies widely. Genotoxic studies are foremost for any occupational exposure studies. Evaluation based on genotoxic parameters is often useful in warranting environmental endowment and occupational health. The present investigation suggests that spray painters under their particular conditions of exposure revealed clear evidence of genotoxicity in exfoliated buccal cell when evaluated by MN test. This shows synergistic effect between habitual usage and occupational exposure. Besides elevated MN frequency, organic solvents exposed spray painters exhibited raised prevalence of other NAs like BN, BE and KL. These abnormalities occur at an elevated level in response to cellular injury. Thus the results of the present study make it clear that the spray painters had a significant increase in cytogenetic damage. These workers may not be aware that they have been exposed to genotoxic agents nor do they know the type and amount of agent to which they have been exposed. Employees, who may be exposed directly or indirectly to paint, need to be made aware of the genotoxic effects and ensure safe and healthy working atmosphere to alleviate the health hazards that they may encounter.

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Table 1: General characteristics of groups studied

Study group		n	Age (years) M ±SD	Average no of cigarettes/ day	Alcohol intake in last 1 yr (g alcohol drinking/day) M ±SD	Duration of employment (years) M ±SD
Controls n = 80	Smokers	24	34.60 ± 2.18	21	-	-
	Non-smokers	18	32.21 ± 1.10	-	-	-
	Alcoholics	21	35.02 ± 3.11	19	83.41 ± 35.23	-
	Non-alcoholics	17	36.21 ± 1.33	-	-	-
Workers n = 94	Smokers	28	33.13 ± 2.12	23	-	10.62 ± 2.05
	Non-smokers	22	30.42 ± 1.99	-	-	7.33 ± 1.53
	Alcoholics	26	34.53 ± 2.44	20	91.32 ± 32.51	9.54 ± 1.71
	Non-alcoholics	18	35.61 ± 3.13	-	-	8.67 ± 0.98

M ± SD = Mean ± Standard Deviation.

Table 2: The frequencies of micronuclei and other nuclear abnormalities in exfoliated buccal epithelial cells of control and exposed subjects

Study group		n	MN (M ±SD)	BN (M ±SD)	BEC (M ±SD)	KLC (M ±SD)
Controls n = 80	Smokers	24	3.18 ± 1.36	5.98 ± 2.28	6.97 ± 1.33	19.52 ±3.65
	Non-smokers	18	1.43 ± 1.08	2.21 ± 2.71	3.12 ± 2.29	10.80 ±1.97
	Alcoholics	21	3.96 ± 0.47	5.16 ± 1.76	6.21 ± 2.81	18.07 ± 2.78
	Non-alcoholics	17	1.08 ± 1.24	2.67 ± 2.27	2.78 ± 1.46	1175 ± 4.54
Workers n = 94	Smokers	28	12.52 ± 0.21*	13.25 ± 2.36**	17.91 ± 4.27*	35.70 ± 1.69*
	Non-smokers	22	8.64 ± 1.32*	9.01 ± 1.52**	13.17 ± 3.76*	24.58 ± 3.50*
	Alcoholics	26	11.59 ± 1.08*	12.67 ± 2.50**	16.94 ± 5.82*	31.83 ± 1.72*
	Non-alcoholics	18	9.16 ± 1.75*	9.85 ± 3.51**	12.57 ± 5.93*	26.42 ± 3.52*

MN= cells with micronuclei; BN= binucleated cells; BEC= broken egg cells; KLC= karyolytic cells *Significantly different, $p < 0.05$; **significantly different, $p < 0.01$(Mann-Whitney test).