Assessment of Genotoxicity Among Rubber Industry Workers Occupationally Exposed to Toxic Agents Using Micronucleus Assay

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Abstract

Background: Occupational and environmental exposures mostly represent complex mixture of genotoxic agents, however there is a wide variation in the specificity of biomarkers. Exploration of correlations among biomarkers contributes to the further progress of molecular cancer epidemiology and to the selection of the optimal biomarkers for the investigation of human exposure to carcinogens. The aim of this study was to assess the potential cytogenetic damage associated with occupational exposure to toxic agents among rubber industry workers by using Micronucleus (MN) assay.

Methods: In the present study 35 occupationally exposed rubber industry workers and 30 controls were investigated for genetic damage. Both the exposed and control individuals were selected from rural areas of South India. Exfoliated Buccal cells were collected from the study population and examined for the presence of MN.

Results: Rubber industry workers showed a significant increase in micronucleated cells when compared to controls with respect to their smoking and drinking habits ($P < 0.05$). The present study suggested that occupational exposure to toxic chemicals in rubber industry can cause genetic damage.

Conclusion: MN formation reflects genetic changes and/or events associated with carcinogenesis. Therefore the results of this study indicate that rubber industry workers may be at the risk of cancer. Therefore, it is important to take appropriate measures to protect the workers from occupational hazards.

Keywords: DNA damage; Micronucleus test; Occupational exposure

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Introduction

Rubber industry uses a broad spectrum of chemicals which are known to be genotoxic [1]. International Agency for Research on Cancer (IARC) has described that rubber industry workers are exposed to aromatic amines, carbon black, Polycyclic Aromatic Hydrocarbons (PAH), nitrosamines and solvents which are known to be genotoxic and the cancer mortality has increased threefold. High risk of lung, gastrointestinal, laryngeal cancer and leukemia were reported in rubber industry workers [2-5].

Genomic damage is probably the most important fundamental cause of developmental and degenerative diseases. Genomic damage is produced by environmental exposure to genotoxins and life style factors like alcohol, smoking, drugs and stress [6-8]. Hence, biomonitoring of human genotoxicity induced by complex occupational or environmental exposure to genotoxic agents is highly essential. 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 and micronucleus assay has been found to be an excellent tool to serve as a genotoxicological biomarker [9, 10].

Micronuclei (MN) are small chromatin bodies that appear in the cytoplasm by the condensation of

acentric chromosome fragments or by whole chromosomes, lagging behind the cell division. Thus, it is the only biomarker that allows the simultaneous evaluation of both clastogenic and aneugenic effects in a wide range of cells, since they are easily detected in interphase cells. In this way, the analysis of MN in epithelial cells has shown to be a sensitive method for monitoring genetic damage in human populations [10-12].

Rubber industry workers are engaged in different production process like compounding and mixing, component preparation, product building, curing and final finish which involves the exposure to number of chemicals, high temperature and personal hygiene varies in their workplace [13, 14]. There is no sufficient information for the assessment of genotoxicity concerning the occupational exposure to rubber industry. Considering these the present study aimed to investigate the genotoxic effects in buccal cells in an exposed population of rubber industry in South India using MN test.

Materials and Methods

A total of 65 individuals (35 rubber industry workers and 30 controls) were analyzed in this study. The workers in the age group 28-50 years with varying exposure duration (5-25 years) were included in the study. The experimental group was further divided as smokers, non-smokers, alcoholics and non-alcoholics. The participation of each subject was voluntary and the subjects could withdraw at any time during the study. Subjects with both smoking and alcohol consumption were excluded from the study. The control group was selected from the general population with no history of exposure to any kind of toxic chemicals, any serious medical problem and intake of drugs or other therapeutic medicines (at least from the past one year from the day of sampling). Controls were matched by age and sex to the exposed workers.

All participants signed a written informed consent before sampling. Complete information regarding sex, age, marital status, medical history, life style along with the occupational history, duration of exposure, protective measures used, smoking and alcohol consumption habit etc. was enquired from the workers and recorded. In all cases, individuals who smoked more than five cigarettes per day for at least one year and those who consumed 120gm of alcohol/day were considered as smokers and alcohol consumers. The whole study population was informed about the aim, risks and methodology details of the study through the informed consent, which was obtained from all individuals. The study was conducted in accordance with the principles for human experience as defined by the Helsinki declaration.

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 [15]. Prior to BC collection, the mouth was rinsed thoroughly with water to remove any unwanted debris. BC samples were obtained by scrapping both cheeks using a wooden spatula. The cells were collected in tubes containing 3ml sterile saline. Exfoliated cells were stained by the Feulgen reaction and counter stained with fast green as previously described by Stich *et al*. [16] with some minor modifications. The cytoplasm was stained a pale blue-green and the nuclei and micronuclei purple red (Figure 1). A total of minimum 2000 cells per individuals were scored for analysis of micronuclei. The slides were randomized and scored by a single observer.

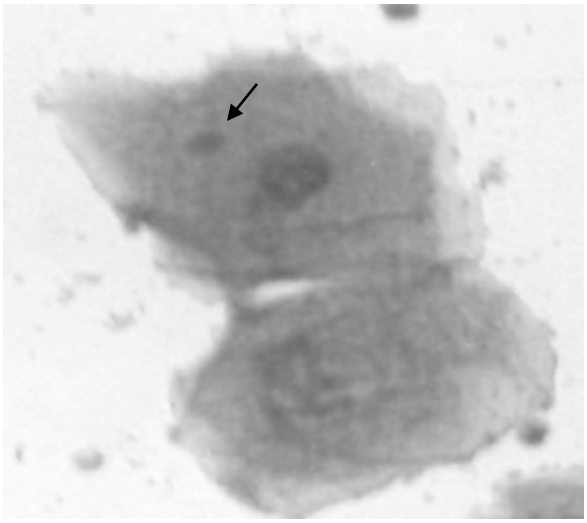

Figure 1. Micronucleated cell (arrow), 40 x magnification, Feulgen fast green stain

Table 1. General characteristics of groups studied

	Study group	N	Age (years) mean ±SD	Average no of cigarettes/ Day(mean±SD)	Alcohol intake in last 1 yr (g alcohol drinking/day) mean ±SD	Duration of employment (years) mean ±SD
Controls (n=30)	Smokers	19	46.15 ± 5.16	9.0±0.94	-	-
	Non-smokers	11	47.90 ± 2.16	-	-	-
	Alcoholics	16	47.31 ± 3.01	-	171.0±16.9	-
	Non-alcoholics	14	46.21 ± 5.53	-	-	-
Workers (n=35)	Smokers	18	47.38 ± 2.78	9.1±0.61	-	17.5±3.8
	Non-smokers	17	47.58 ± 1.62	-	-	16.8±2.0
	Alcoholics	20	47.35 ± 2.64	-	172.3±17.54	18.05±2.1
	Non-alcoholics	15	47.66 ± 1.71	-	-	19.66±1.7

Table 2. The mean frequency of micronuclei in exfoliated buccal epithelial cells of rubber industry workers and controls

Study group		N	MN (mean±SD)
Controls (n=30)	Smokers	19	1.31±0.67
	Non-smokers	11	1.18±0.75
	Alcoholics	16	0.93±0.57
	Non-alcoholics	14	0.57±0.64
Workers (n=35)	Smokers	18	2.55±1.04*
	Non-smokers	17	2.0±0.93*
	Alcoholics	20	1.90±0.64*
	Non-alcoholics	15	1.53±0.83*

MN=cells with micronuclei *significantly different with their respective controls, $p<0.05$.

The following criteria for MN analysis were used in oral epithelial cells. An MN must be less than one third the diameter of the main nucleus; must be on the same focal plane; must have the same color, texture and refraction as the main nucleus; must have a smooth oval or round shape; and must be clearly separated from the main nucleus.

All calculations were performed using Windows statistical package, SPSS, version 11.5 (IL, USA). Student's t-test was used for age and time comparisons. Mean values and standard deviations were computed for the scores and the statistical significance ($P < 0.05$) of effects (exposure, smoking, alcohol consumption and age) was determined using Analysis of Variance (ANOVA).

Results

The general characteristics of the population studied are presented in Table 1. The age, sex, smoking and alcohol consumption status distributions were not significant among exposed workers and controls. The Micronuclei (MN) frequency was studied in 35 rubber industry workers and in 30 controls. Workers showed a significant induction of MN when compared with controls ($p< 0.05$). Individuals belonging to the exposed as well as control groups with smoking habit and alcohol consumption showed an increase in MN frequency (2.55 and 1.90) Vs. (1.31 and 0.93) when compared to the non-smokers and non-alcoholics (2.0 and 1.53) Vs. (1.18 and 0.57). A highly significant increase ($p<0.05$) in MN frequency was observed in smokers and alcoholics when compared to all other groups and subgroups (Table 2).

Discussion

Occupational exposure to hazardous chemicals is common in industries using solvent based materials as well as in indoor environments where people are exposed to volatile organic compounds [17], many

of which has the potential to cause cancer. Rubber manufacturing process is extremely complicated and involves exposure to large variety of agents in all three physical states and by dermal contact and inhalation [18].

Buccal cells are the first barrier for the inhalation and are capable of metabolizing proximate carcinogens to reactive products [19, 20]. Approximately 90% of human cancers originate from epithelial cells [21]. Therefore, it could be argued that oral epithelial cells represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion. In the early studies from the 1980s, exfoliated buccal epithelial cells were used to evaluate the genotoxic effects and found to be an efficient tool for biomonitoring studies [22, 23]. The MN assay in buccal cells was also used as a biomarker in several genotoxicological studies [24-27].

The detection of an elevated frequency of micronuclei in smokers indicates increased risk of cancer [28-30]. Similarly in the present study, a significant increase in the MN frequency was observed among the exposed group with smoking and alcohol consumption habit. Our findings are in agreement with those of the Sram *et al*. [29] who detected a significant increase in the frequency of micronuclei in the lymphocytes of 1, 3-butadiene-exposed workers. An increase in chromosomal aberration and Sister Chromatid Exchange (SCE) in workers from rubber industry was also reported by Sorsa *et al*. [30] and Sasiadek [31].

Smoking and alcohol consumption showed an influence on MN frequency in different occupational studies [32]. Cigarette smoking is one of the factors that may influence the rate of cytogenetic damage [33-35]. Smoking is also reported to increase the MN frequency in buccal cells [36, 37]. Our findings indicate that cigarette smoking significantly increases the frequencies of MN in both exposed and non-exposed workers, these are consistent with recent reports suggesting an association between smoking and occupational exposure [38]. More studies are needed to investigate interaction between an occupational exposure and smoking.

Conclusion

Our findings conclude that rubber industry exposure induce genotoxic effect in buccal epithelial cells in the workers and can be taken as an indication that these individuals have increased cancer risk. An intervention study with a large sample size would be needed before any definitive conclusions can be drawn.

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Conflict of Interest

There is no conflict of interest in this study.

Authors' Contribution

Gem Gemitha and Sellappa Sudha contributed to the study design and data analysis, literature review and writing-up process. Gem Gemitha analyzed the data and wrote the paper. Both authors read and approved the final manuscript.

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