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Research Paper

Wood Dust Induced Genotoxicity as an Occupational Hazard in Carpentry Workers

MEDICAL SCIENCE

KEYWORDS : Wood dust, chromosomal aberration assay, genotoxicity

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ABSTRACT

To evaluate the extent of genetic damage caused by the wood dust exposure in carpentry workers of Bhopal-Madhya Pradesh, blood samples from 25 carpentry workers and 25 controls were collected after written informed consent and analyzed for chromosome aberrations. Genotoxicity was studied by chromosomal aberration assay in cultured peripheral blood lymphocytes of carpentry workers. To analyze the results, statistical analysis was performed using Student's t-test. Chromosomal aberrations were found to be statistically higher in the wood dust exposed persons than the control group. The increased percentile of chromosomal damage in the carpentry workers was directly related to their exposure to wood dust. The findings of this investigation indicate that carpentry workers seem to be facing the occupational hazard of genotoxicity due to inhalation of wood dust and are at an elevated risk of diseases that can be caused by the chromosomal damage.

INTRODUCTION

Wood work in carpentry shops for manufacture of furniture has been a major industry since several decades throughout the world. Every year near about 1700 million m^3 of wood is being harvested for industrial purposes[1]. In the year 2000, about 3.5 million workers were occupied in furniture industry[2]. Processing of wood for a variety of uses generates wood dust, a complex mixture of cellulose, polyoses, lignin and a variable number of polar, non-polar and water soluble compounds[1]. Wood dust becomes a potential health problem when wood particles from processes such as sanding, cutting, drilling, chipping, sawing or turning to shape wood becomes air borne. These particles cause mucosal, allergic and non-allergic respiratory symptoms and even cancer when they get deposited in nose, throat and other airways. Review of literature reveals that occupational exposure to wood dust may result in cancer and other health hazards[3-7]. Induction of colorectal cancer[8], cancer of lung, pharynx and stomach[9] and adenocarcinoma of nasal cavity[10] has been confirmed from several studies on human populations exposed to wood dust.

Genetic effects have important health implications for the induction of cancer. Hence, the undesirable health effects caused by wood dust exposure in humans are of special concern. Genetic biomonitoring of populations exposed to potential carcinogens is an early warning system for genetic diseases or cancer. It also allows identification of risk factors at a time when control measures could still be implemented[11]. In order to ascertain occupational exposure to wood dust, measurement of biomarkers such as chromosomal aberrations can be considered suitable.

So far, there is limited data available on the genotoxicity and biochemical alterations induced by occupational exposure to wood dust from Central India. Hence, in the present study, the genotoxicity related to wood dust exposure was evaluated using chromosomal aberration (CA) assay. The CA analysis is a method used to assess exposure to genotoxins that is internationally recognized.

Cytogenetic biomarkers, being the most frequently used end point in human biomonitoring studies, are used extensively to assess the impact of environmental, occupational and medical factors on genomic stability. The present study was one such attempt to determine genotoxicity in wood dust exposed workers of Bhopal-Madhya Pradesh by performing chromosomal aberration assay in their peripheral blood lymphocytes.

MATERIALS AND METHODS

Study Groups

The study group included twenty five healthy workers of the carpentry shops in Bhopal, India. It was made sure that all the workers are non-smokers and not exposed to any kind of radiation or other hazardous chemicals. All the workers were included after their informed consent as per the guidelines of Institutional Ethics Committee. Twenty five volunteers were included in the study as the controls, following the same criteria. All the wood workers and the controls were males and belonged to the age group of 25-45.

Sample Collection

A 2ml of peripheral blood sample was collected from each person of the exposed and the control group once with the help of vacutainers and all the samples were immediately transported to the laboratory for culturing.

Lymphocyte Culture

Lymphocyte cultures were set according to the standard protocol given by Moorhead et al[12]. Peripheral venous blood samples of wood dust workers were aseptically transferred into sterile culture bottles with 5-8ml of RPMI-1640 medium (Sigma,USA), supplemented with L- glutamine, 10% fetal bovine serum (Himedia Labs, India), Penicillin - streptomycin solution (Invitrogen, USA) and phytohaemagglutinin (Himedia Labs, India). The cultures were incubated in a CO_2 incubator for 72 hours. 50µl colchicine was added to each tube at the completion of 70 hours to arrest the cells at metaphase. After 72 hours of incubation, the cell suspensions were centrifuged for 10 minutes at 1000 rpm. The supernatant was discarded and the pellets were treated with hypotonic solution (0.075M KCl) by gentle flushing and cyclomixed. The centrifuge tubes were incubated again at 37°C for 45 minutes. The tubes were again centrifuged carefully at 1000 rpm for 15 minutes. The supernatant was removed and 5-8 ml of freshly prepared pre-chilled Cornoy's fixative was added to the pellets while mixing on cyclomixer. The tubes were allowed to stand overnight and washed with freshly prepared pre-chilled Cornoy's fixative repeatedly for 3-4 times. The slides were pre-

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pared by air drop method and stained with 1% Giemsa stain[13]. Chromosomal aberrations were recorded on well spread metaphases and at least thirty metaphases were screened per sample.

Statistical Analysis

Student's T test was performed on the means of chromosomal aberrations in the control and the exposed group with the help of PRISM version 4 software on PC.

RESULTS

Chromosomal aberrations were found to be higher in the wood dust exposed persons than the controls. The mean percentage of total aberrant metaphases in the exposed group was found to be 24.05 ± 2.25 which was statistically higher (P<0.01) than mean percentage of the total aberrant metaphases in the control group (8.46 ± 1.05).

The chromosomal aberrations observed in the exposed group included chromosome-type and chromatid-type aberrations. The chromosome-type aberrations comprised of dicentric chromosomes and acentric fragments (Figure 1). The chromatid-type of aberrations included terminal deletions and chromatid breaks (Figure 2). All these types of aberrations were found to be statistically higher in the exposed group than that in controls. The incidences of these chromosomal aberrations are given in Table 1.

Table 1: Mean percentage of different chromosomal aberrations in the exposed and the control group.

Type of aberration	Control Group (Mean ± S.E.)	Exposed Group (Mean ± S.E.)
Dicentric chromosomes	0.00 ± 0.00	2.25 ± 0.22*
Acentric fragments	1.02 ± 0.02	3.04 ± 0.08*
Terminal deletions	3.00 ± 0.60	6.04 ± 0.24*
Chromatid breaks	2.80 ± 0.02	09.50 ± 1.06*

*Significantly increases when compared to the control group (P value <0.001)

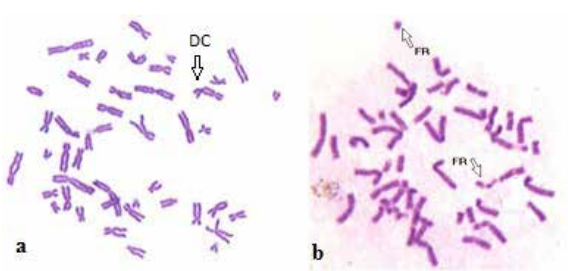

Figure 1: a-Metaphase showing dicentric chromosome (DC), b-Metaphase showing acentric fragment (FR)

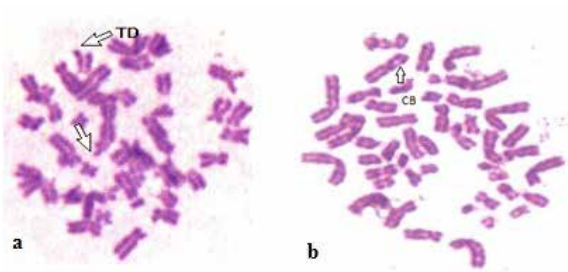

Figure 2: a-Metaphase showing terminal deletion (TD), b-Metaphase showing chromatid break (CB)

DISCUSSION

The mean percentage of total aberrant metaphases in the exposed group was found to be statistically higher than that of control group. This is an evidence of genotoxicity in the workers exposed to wood dust. Genotoxicity of wood dust has been studied in several short-term tests using a variety of end points. A study on human epithelial cell line A549 incubated with different wood dust extracts showed detectable DNA damage[2]. Besides, beech wood extract was found to be mutagenic when tested on *Salmonella typhimurium* by Ames assay[14] Genotoxicity of wood dust, when analysed by micronucleus test (MNT) showed significant induction of micronuclei in mice treated with birchen wood dust[15], bass wood[16] and rats dosed with beech wood dust[17].

Genotoxicity assessment in carpenters using comet assay[1,18,19], MNT in buccal cells[20], MNT in peripheral blood lymphocytes[15,16,21], chromosomal aberrations[22] and sister chromatid exchanges[21] revealed a significant genetic effect from occupational exposure to wood dust. The generation of oxygen-free radicals, lipid peroxidation and activity of superoxide dismutase (SOD) were determined in a study to analyse dose–effect relationship of wood dust and genotoxicity. The activity of SOD was significantly lowered and lipid peroxidation was found to be higher in exposed subjects in comparison to controls[16].

Chromosomal aberrations were found to be statistically higher in the exposed group than the control group. The role of some chemicals in inducing DNA double-strand breaks that, if not repaired, result into structural chromosomal aberrations during cell division has been established[23]. Besides, measuring the frequency of chromosomal damage in humans exposed to environmental clastogens has been a priority in public health studies for decades and an increased level of chromosomal aberrations in population groups is currently interpreted as an evidence of genotoxic exposure and early biologic effects on DNA[24]. Significantly higher percentage of chromosomal aberrations in the exposed individuals of the present study can be therefore attributed to their exposure to the toxic chemicals released with the wood dust. As the lesions induced by chemicals are mostly S phase dependent for expression in the subsequent divisional cycle, the damaged T lymphocytes may remain circulating for long periods and these aberrations can be observed only if the cells are stimulated to divide *in* vitro[25]. In general, the types and frequencies of induced chromosomal aberrations depend on the type of mutagen exposure and the cell cycle stage at the time of exposure. Therefore, the increased structural chromosomal aberrations in the carpentry workers of the present study can be attributed to their routine exposure to the different chemicals that are liberated from different woods.

Our findings are supported well by the results of the studies carried out by Rekhadevi *et al.* (2009)[26]. They observed a statistically significant increase in mean DNA damage by comet assay, micronuclei frequency in buccal cells as well as peripheral blood lymphocytes and frequency of chromosomal aberrations in the exposed workers when compared to controls.

Chromosome-type aberrations are formed in the G_0 stage by a mechanism where apurinic or apyrimidinic sites are converted into strand breaks and misrepaired[27]. Dicentric chromosomes are produced by the interchange between two separate chromosomes when breaks occur in each one early in interphase and sticky ends are formed. The presence of dicentric chromosomes only in the exposed individuals is indicative of severe clastogenic effect of the wood dust exposure. Incidence of acentric fragments was also found to be higher in the exposed group than the controls. As these fragments do not have centromeres, they are consequently lost in the subsequent cell divisions.

The frequency of chromatid-type aberrations in the exposed

group was observed to be higher than the control group. This could be due to the reason that genotoxic chemicals induce a wide variety of lesions in the DNA of lymphocytes in different proportions. Most of the chemically induced aberrations are formed only during the DNA synthesis phase, probably due to replication errors. Exposure to chemical mutagens induces lesions in the DNA of lymphocytes, most of which are removed by cellular repair processes. The non-repaired fraction of lesions gives rise to chromatid-type aberrations during S phase, when the lymphocytes are treated with mitogen *in* vitro[27,25].

CONCLUSION

The findings of the present study suggest that wood dust is an effective clastogen and the workers of carpentry shops are therefore at an elevated risk of diseases that can be caused by the chromosomal damage.

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