Frequency of sister chromatid exchange and chromosomal aberrations in asbestos cement workers

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Abstract

Exposure to asbestos minerals has been associated with a wide variety of adverse health effects including lung cancer, pleural mesothelioma, and cancer of other organs. It was shown previously that asbestos samples collected from a local asbestos factory enhanced sister chromatid exchanges (SCEs) and chromosomal aberrations *in vitro* using human lymphocytes. In the present study, 22 workers from the same factory and 12 controls were further investigated. Controls were matched for age, sex, and socioeconomic state. The peripheral blood lymphocytes were cultured and harvested at 48 hours for studies of chromosomal aberrations and at 72 hours for SCE frequency determinations. Asbestos workers had a raised mean SCE rate and increased numbers of chromosomal aberrations compared with a control population. Most of the chromosomal aberrations were chromatid gap and break types.

Asbestos belongs to a group of naturally occurring magnesium silicate fibrous substances and is widely used in modern industrial society. It is considered to be carcinogenic for several organ systems, and past studies have shown that asbestos exposed workers are at a higher risk of mesothelioma, bronchogenic carcinoma, and other cancers.¹ It has also been found to have mutagenic properties. Chromosomal abnormalities were induced by exposure to asbestos in ovarian cells and lung cells from Chinese hamsters.^{2,3}

Chrysotile has been reported to induce chromosomal abnormalities in cultured human lymphocytes.⁴ Rom *et al*⁵ found that asbestos workers had a raised mean sister chromatid exchange (SCE) rate compared with that of controls. In another study

a slightly higher incidence of chromosomal aberrations in asbestos exposed factory workers compared with controls was found.⁶

The extensive use of asbestos in Indian industry increases the risk of workers to exposure to asbestos and demands a critical evaluation of health hazards from asbestos dust. Our preliminary studies showed enhanced SCE and chromosomal aberrations in human lymphocytes *in vitro* treated with dust from an Indian asbestos cement factory. In the present study the cytogenetic effects of asbestos dust on workers who include both smokers and non-smokers in the same asbestos cement factory and exposed to the same dust have been examined.

Materials and methods

Twenty two asbestos factory workers and 12 controls were studied to assess the frequency of SCE and the incidence of chromosomal aberrations. The controls were of similar age, sex, habits, and socioeconomic state as the factory workers. All subjects were carefully examined and their detailed clinical histories recorded. None of the subjects had taken any drug for at least two months before the sampling of peripheral blood. Subjects were divided into four groups: asbestos exposed smokers, asbestos exposed non-smokers, control smokers and control non-smokers. The mean duration of exposure was 12-0 years.

From each subject peripheral venous blood was collected in a heparinised tube under sterilised conditions. Whole blood lymphocyte culture was done in RPMI-1640 medium supplemented with fetal calf serum (20%), L-glutamine (0.03%), penicillin (100 IU/ml), streptomycin (100 µg/ml), phytohaemagglutinin-M (3%), 5'-bromo-2'-deoxyuridine (5 µg/ml), and blood (0.3 ml). Culture vials were wrapped with aluminium foil and incubated at 37°C in a 5% CO₂ atmosphere.

Cultures were harvested at 48 hours to study chromosomal aberrations and at 72 hours for SCE analysis. Three hours before harvesting, colchicine (0.1 µg/ml) treatment was given to arrest the cells in metaphase. The culture was centrifuged at 1200 rpm for 10 minutes. The supernatant was discarded and pellets were resuspended in 0.075 M KCl and incubated at 37°C for 20 minutes. After further

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centrifugation of the material for 10 minutes at 1200 rpm, the supernatant was discarded and pellets were fixed in fresh chilled methanol: acetic acid (3:1) mixture. Slides were prepared by the flame dry method. For differential staining slides were treated with Hoechst 33258 (50 µg/ml) for 15 minutes, exposed to bright light for two hours in the presence of 2 standard salt concentrations, then stained with Giemsa stain (4%) in phosphate buffer (pH 6.8). Coded slides were scored to avoid bias. One hundred well spread metaphases were scored for chromosomal aberrations and 50 well spread metaphases with good differentiation were scored for SCE analysis for each subject. Statistical analysis was by student's *t* test.

Results

It is evident (table 1) that the aberrant metaphase percentage was significantly higher ($p < 0.01$) in exposed smoker and non-smoker groups in comparison with their respective controls. Chromosomal aberrations were of chromatid gap and break types. Mitotic index was low though not significantly so in both groups. In both of the exposed groups mean

SCE/cell was increased significantly ($p < 0.001$) without affecting mitotic index and cell cycle kinetics (table 2).

A significantly higher SCE/cell in asbestos exposed smokers compared with the exposed non-smokers was found (table 3). The variation in the magnitude of chromosomal aberrations among both exposed smoker and exposed non-smoker groups was not significant. The incidence of asbestosis in the asbestos exposed smoker group was 72.8% and in the non-smoker group 27.2%.

Discussion

A significant increase in chromosomal aberrations and mean SCE/cell in both the exposed groups indicate that exposure to asbestos may induce undesirable genetic damage in an occupational population. The higher mean SCE/cell in asbestos exposed smokers compared with exposed non-smokers may be due to a synergistic action of asbestos and smoking (table 3). The results are in agreement with those of other workers.⁷ The increased SCE rate due to cigarette smoking is in accordance with earlier reports.⁸ The highest frequency of SCEs was found

Table 1 Chromosomal aberrations in asbestos factory workers

Subjects	Age (y; mean (SD))	Duration of exposure (y; mean (SD))	Chromosomal aberrations				
			No of cells scored	Mitotic index (mean (SD))	Aberrant metaphase (%; mean (SD))	Chromatid gap (%; mean (SD))	Chromatid break (%; mean (SD))
Exposed smokers (n = 11)	34.1 (2.4)	12.0 (0.3)	1100	2.14 (0.82)	4.09 (1.51)**	2.05 (1.20)	2.04 (1.21)
Exposed non- smokers (n = 11)	34.0 (2.3)	12.0 (0.4)	1100	2.90 (0.71)	3.54 (1.21)**	1.76 (1.00)	1.78 (0.9)
Control smokers (n = 6)	33.1 (1.9)	0	600	3.90 (0.98)	1.50 (0.54)	0.60 (0.5)	0.90 (0.7)
Control non- smokers (n = 6)	34.0 (2.0)	0	600	3.94 (0.25)	1.30 (0.81)	0.50 (0.4)	0.80 (0.7)

** $p < 0.01$.

Table 2 Sister chromatid exchange frequency in asbestos factory workers

Subjects	Age (y; mean (SD))	Duration of exposure (y; mean (SD))	Sister chromatid exchange				Cell cycle kinetics (%)		
			No of cells scored	Mitotic index (mean (SD))	SCE/cell (mean (SD))	SCE range	1st	2nd	3rd
Exposed smokers (n = 11)	34.1 (2.4)	12.0 (0.3)	550	5.39 (0.72)	8.16 (0.45)***	3-16	30.39 (6.30)	55.98 (5.53)	12.50 (4.11)
Exposed non- smokers (n = 11)	34.0 (2.3)	12.0 (0.4)	550	5.44 (0.60)	6.63 (0.50)***	3-12	29.20 (6.63)	57.45 (9.62)	10.15 (1.61)
Control smokers (n = 6)	33.1 (1.9)	0	300	5.80 (0.78)	5.73 (0.16)	3-9	33.64 (4.32)	57.86 (3.34)	8.74 (1.72)
Control non- smokers (n = 6)	34.0 (2.0)	0	300	5.86 (0.80)	3.61 (0.14)	3-6	33.48 (5.18)	56.44 (4.03)	10.05 (3.47)

*** $p < 0.001$.

Table 3 Chromosomal aberrations, sister chromatid exchange frequency, and incidence of asbestosis in asbestos factory workers

Exposed non-smokers				Exposed smokers			
Subject No	Chromosomal aberration (%)	SCE/cell (mean (SD))	Asbestosis	Subject No	Chromosomal aberration (%)	SCE/cell (mean (SD))	Asbestosis
1	2	5.88 (2.13)	A	1	4	8.34 (1.36)	A
2	5	6.56 (2.17)	A	2	3	8.10 (1.57)	P
3	4	7.26 (1.73)	P	3	3	7.52 (1.56)	A
4	4	7.15 (2.20)	P	4	5	8.38 (2.15)	P
5	3	6.08 (1.95)	A	5	3	8.52 (1.68)	A
6	6	7.16 (1.55)	P	6	2	8.66 (1.93)	P
7	4	7.04 (1.90)	A	7	3	8.12 (2.42)	P
8	3	6.10 (2.30)	A	8	7	8.70 (1.18)	P
9	3	6.98 (1.34)	A	9	5	7.58 (1.67)	P
10	3	6.40 (1.62)	A	10	6	8.48 (1.57)	P
11	2	6.36 (1.74)	A	11	4	7.46 (2.40)	P
Mean (SD)	3.54 (1.21)	6.63 (0.50)			4.09 (1.51)	8.16 (0.45)***	

***p < 0.001.

P = Present; A = absent.

in exposed smokers and the lowest in control non-smokers. The higher incidence of asbestosis in the asbestos exposed smoker group (72.8%) in comparison with the asbestos exposed non-smokers group (27.2%) suggests that smoking may act synergistically to enhance asbestosis in the smoker group. These results are analogous to lung cancer risk among insulators where most cases occur in those insulators who smoke. The exact mechanism resulting in the production of SCE is not well established, although SCE analysis has been adopted as a sensitive indicator of genetic damage.⁹

Marked variation in SCE frequencies has been reported among people with various types of cancer.¹⁰ Also, an increase in SCE level has been found in cohort studies in those persons who are at a higher risk of cancer due to occupational or environmental exposure to a wide variety of both mutagens and carcinogens.¹¹ It can be inferred from the above studies that these chromosomal abnormalities may be an indication of the risk in the development of cancer in exposed workers. Further study is required, however, to establish that increased SCE and chromosomal aberrations are associated with development of lung cancer in workers exposed to asbestos.

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