SISTER CHROMATID EXCHANGE FREQUENCY AND CHROMOSOMAL ABERRATIONS IN ASBESTOS FACTORY WORKERS

QAMAR RAHMAN, Ph.D. • F. Nahid,* M.Sc. • A. K. Jain,* Ph.D • S. S. Ayarwal,* M.D.

Industrial Toxicology Research Centre, Post Box No. 80
Lucknow-226 001, India
*ICMR Centre for Advanced Research in Genetics, Dept. of Medicine
K.G's Medical College, Lucknow—226 003, India

INTRODUCTION

Asbestos, belongs to a group of naturally occurring magnesium silicate fibrous substances. It has a wide variety of uses in modern society. It is considered to be a carcinogenic for several organ systems.[13] Past study reveals that asbestos exposed workers have an increased risk to develop mesothelioma, bronchogenic carcinoma and other cancers.[3,4] It has also been found to have mutagenic properties. Sincock and Seabright[14] first demonstrated that Chinese hamster ovary cells exposed to chrysotile and crocidolite asbestos showed the occurrence of chromatid and chromosomal changes. It has also been reported that chrysotile produced chromosomal aberrations in cultured Syrian hamster embryo cells in dose related manner.[8]

Chrysotile (UICC) variety has already been reported to induce chromosomal abnormalities in cultured human lymphocytes.[17] Rom et al.[12] found that asbestos workers had an elevated mean sister chromatid exchange (SCE) rate compared to that of controls. In another study slightly higher incidence of chromosomal aberrations in asbestos exposed factory workers was observed.[16]

An Indian variety of chrysotile asbestos has also been found to induce chromosomal aberrations and sister chromatid exchange in Chinese hamster ovarian cells in vitro.[1,2]

In India extensive use of asbestos in various occupations leading to an increased risk of asbestos exposure to workers demands a critical evaluation of asbestos dust. Therefore, present work was undertaken to evaluate the cytogenetic effects of asbestos dust on workers in an asbestos cement factory. This preliminary report may be helpful to determine genotoxic effects of asbestos dust exposure on human population.

MATERIALS AND METHODS

22 factory workers as well as 12 controls were studied to assess the frequency of sister chromatid exchange and the incidence of chromosomal aberrations. The controls were of similar age, sex, having similar habits and socio-economic status. All subjects were carefully examined and detailed clinical history was taken. No subject had taken any drug for at least two months prior the sampling of peripheral blood. All subjects were divided in 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 heparinized tube under sterilized conditions. Whole blood lymphocyte culture was done in RPMI-1640 medium supplemented with fetal calf serum (20%), L-glutamine (0.03%), penicillin (100 I U/ml), Streptomycin (100 µg/ml), Phytohemagglutinin-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 5% CO_2 atmosphere.

Cultures were harvested at 48 hours to study chromosomal aberrations and at 72 hours for SCE analysis. 3 hours prior to harvesting colchicine (0.1 µg/ml) treatment was given to arrest the cells in metaphase. Centrifugation of the culture was done at 1200 rpm for 10 minutes. Supernatant was discarded and pellets were resuspended in hypotonic solution (0.075 M KC1) and incubated at 37°C for 20 minutes. Again centrifugation of the material was done for 10 minutes at 1200 rpm, the supernatant was discarded and pellets were fixed in fresh and chilled fixative methanol and acetic acid (3:1). Slides were prepared by flame dry method. For differential staining slides were stained with Hoechst 33258 (50 µg/ml) for 15 minutes and then exposed to bright sunlight for 2 hours in the presence of 2 SSC followed by staining with Giemsa (4%) in phosphate buffer (pH 6.8). Coded slides were scored to avoid scoring bias. 100 well spread metaphases were scored for chromosomal aberrations and 50 well spread metaphases with good differentiation were scored for SCE analysis for each subject. Students' 't' test was used for statistical analysis.

RESULTS AND DISCUSSION

In the present study all the subjects were of similar age group. It is evident from Table I that the aberrant metaphase percentage was significantly higher ($p < 0.01$) in exposed smoker and exposed non-smoker groups in comparison to their respective control. Chromosomal aberrations were of chromatid gap and break type (Figure 1). Mitotic index was low in both the groups but not significantly. In both the exposed groups mean SCE/cell was elevated significantly ($p < 0.001$) without affecting mitotic index and cell cycle

Table I

Chromosomal Aberrations in Asbestos Factory Workers
The Values Represent Mean ± SD *(P / 0.01)

Subjects	Age (yr) Mean ± S.D.	Duration of exposure (yr) Mean±S.D.	Chromosomal aberrations				
			No. of cells scored	Mitotic index Mean±S.D.	Aberrant Metaphase (%) Mean±S.D.	Chromatid gap (%) Mean±S.D.	Chromatid break (%) Mean±S.D.
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

n = Number of subject

* ρ / 0.01

kinetics (Table II). It indicates that asbestos exposure may induce undesirable genetic damage in occupational populations.

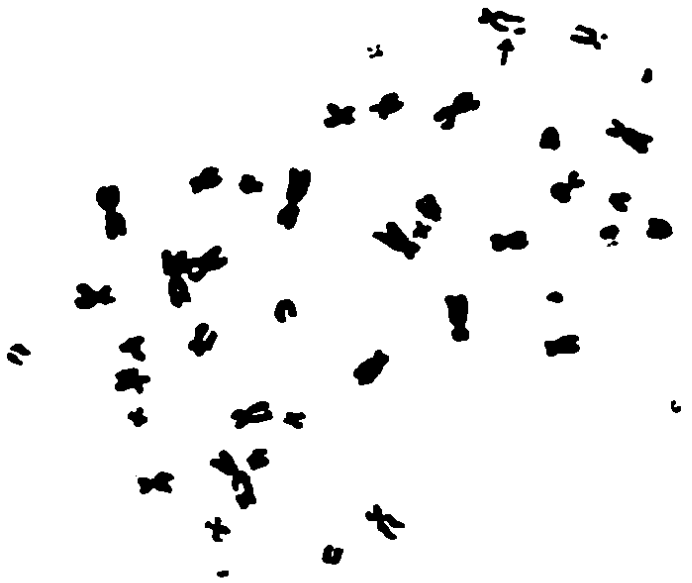


Figure 1. Cell with chromatid break in an exposed non-smoker subject.

The higher SCE/cell (Figure 2) in asbestos exposed smokers (8.16±0.45) in comparison to exposed non-smokers (6.63±0.50) (p / 0.001) may be due to synergistic action of asbestos and smoking (Table III). The results are in agreement with other workers.[6,12] The variation in the magnitude of chromosomal aberrations among both exposed smoker (4.09±1.51) and exposed non-smoker groups (3.54±1.21) was not significantly different. The elevation of SCE rate due to cigarette smoking is in accordance with the earlier reports.[7,10] The highest SCE frequency was observed in exposed smokers and lowest in control non-smokers. These results are analogous to lung cancer risk among insulators where the vast majority of cases occur in those insulators who smoke. The exact mechanism involved in the production of SCE is not well established. However, SCE analysis has been adopted as sensitive indicator of genetic damage.[11] The higher incidence of asbestosis in asbestos exposed smoker group (72.2%) in comparison to asbestos exposed non-smokers group (27.2%) (Table III) suggests that smoking may act synergistically to enhance asbestosis in the smoker group.

Marked variation in SCE frequencies have also been reported among individuals with several different types of cancer.[5,9,18] In addition, increase in SCE level has reportedly been found in cohort studies in those individuals who are at a higher risk of cancer due to occupational or environmental exposure to a wide variety of both mutagens as well as carcinogens.[15] We conclude that the evaluation of SCE per cell and higher chromosomal aberrations may point out the

Table II

Sister Chromatid Exchange Frequency in Asbestos Factory Workers
The values are expressed as mean ± SD *(P / 0.001).

Subjects	Age (yr) Mean ± S.D.	Duration of exposure(yr) Mean ± S.D.	Sister chromatid exchange						
			No.of cells Scored	Mitotic index Mean±S.D.	SCE/cell Mean±S.D.	SCE range	Cell cycle kinetics (%) Ist	IInd	IIIrd
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 smoker (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

n = Number of subject

* p / 0.001

Figure 2. Cell showing sister chromatid exchanges in an exposed smoker subject.

risk of developing cancer in asbestos exposed workers. However, further study is required to establish the fact that higher SCE and chromosomal aberrations are associated with development of lung cancer in asbestos exposed workers.

REFERENCES

1. Babu, K.A., Lakkad, B.C., Nigam, S.K., Bhatt, D.K.,Karnik, A.B., Thakore, K.N., Kashyap, S.K., Chatterjee, S.K.: *In vitro* cytological and cytogenetic effects of an Indian variety of chrysotile asbestos. *Environ. Res.*, 21:416-422. (1980).
2. Babu, K.A., Nigam, S.K., Lakked, B.C., Bhatt, D.K., Karnik, A.B., Thakore, K.N., Kashyap, S.K., Chatterjee, S.K.: Effect of chrysotile asbestos (AP-1) on sister chromatid exchange in Chinese hamster ovary cells. *Environ. Res.* 24:325-329. (1981).
3. Becklake, M.R.: Asbestos related diseases of the lungs & other organs; their epidemiology and implications for clinical practice. *Am. Rev. Respir. Dis.* 114:187-227. (1976).
4. Craighead, J.E., Mossman, B.T.: The pathogenesis of asbestos associated diseases. *N. Engl. J. Med.* 306:1446-1455. (1982).
5. *Differentiation and neoplasm.* Vol II pp. 93-101—R.G. Mackinell, M.A. Berardino and M. Blumenfeld et al. eds. Springer-verlag, Berlin. (1981).
6. Kelsey, K.T., Christiani, D.C., Little, J.B.: Enhancement of benzo(a)pyrene-induced sister chromatid exchanges in lymphocytes from cigarette smokers occupationally exposed to asbestos. *JNCI* 77:321-327. (1986).
7. Lambert, B., Lindblad, A., Nordenskjold, M., Werelius, B.: Increased frequency of sister chromatid exchanges in cigarette smokers. *Hereditas.* 88:147-149. (1978)
8. Lavappa, K.S., Fu, M.M., Epstein, S.S.: Cytogenetic studies on chrysotile asbestos. *Environ. Res.* 10:165-173. (1975).

Table III

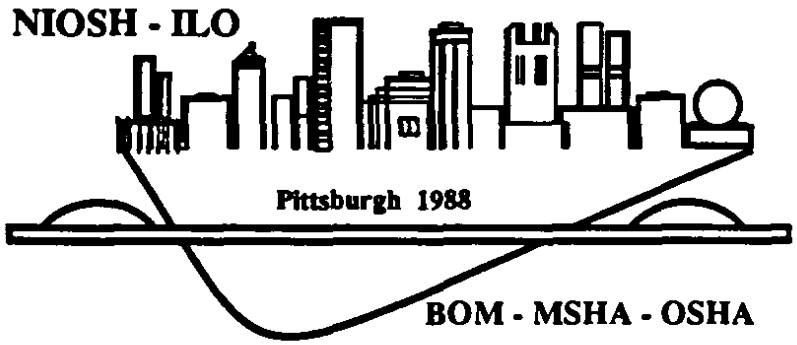
Chromosomal Aberrations, Sister Chromatid Exchange, Frequency and Incidence of Asbestosis in Asbestos Factory Workers
The values represent mean ± SD *(P / 0.001).

Exposed non smokers					Exposed smokers				
S.No.	Chromosomal aberration (%)	SCE/Cell mean±S.D.	Asbestosis	Incidence of asbestosis (%)	S.No.	Chromosomal aberration (%)	SCE/Cell mean±S.D.	Asbestosis	Incidence of 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	27.2	6.	2	8.66±1.93	P	72.7
7.	4	7.04±2.90	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± S.D.	3.54±1.21	6.63±0.50				4.09±1.51	8.16±0.45*		

P = Present
A = Absent
* = (P/ 0.001)

9. Livingston, G.K., Cannon, L.A., Bishop, D.T. et al: Sister chromatid exchanges: Variation by age, sex, smoking and breast cancer status. *Cancer Genet. Cytogent.* 9:289-299. (1983).
10. Murthy, P.B.: Frequency of sister chromatid exchanges in cigarette smokers. *Hum. Genet.* 52:343-345. (1979).
11. Perry, P., Evans, H.J.: Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature* 258:121-125. (1975).
12. Rom, W.N., Livingston, G.K., Casey, K.R., Wood, S.D., Egger, M.J., Chiu, G.L., Jerominski, L.: Sister chromatid exchange frequency in asbestos workers *JNCI.* 70:45-48. (1983).
13. Rom, W.N., Palmer, P.E.S.: The spectrum of asbestos related diseases. *West. J. Med.* 121:10-20. (1974).
14. Sincock, A., Seabright, M.: Induction of chromosome changes in Chinese hamster cells by exposure to asbestos fibres. *Nature* 257:56-58. (1975).
15. Sorsa, M.: *Monitoring of sister chromatid exchange and micronuclei as biological endpoints.* IARC Sci Publ. No. 59. pp. 339-350—Lyon, France. (1984).
16. Srb, V.E., Kucova, J.M. Musil: Testing genotoxic activity in exposure to asbestos. 1. Cytogenetic examination of lymphocytes of human peripheral blood. *Proc. Lek.* 36:175-178. (1984).
17. Valerio, F., DeFerrari, M., Ottaggio, L., Repetto, E., Santi, L.: *Cytogenetic effects of Rhodesion chrysotile on human lymphocytes in vitro.* IARC Sci. Publ. No. 30. pp. 485-489. Lyon, France. (1980).
18. Wiencke, J.K., Vosika, J., Johnson, P. et al: Differential induction of sister chromatid exchange by chemical carcinogens in lymphocytes cultured from patients with solid tumors. *Pharmacology.* 24:67-73 (1982).

Proceedings of the VIIth International Pneumoconioses Conference | *Part*
Transactions de la VIIe Conférence Internationale sur les Pneumoconioses | *Tome*
Transaciones de la VIIa Conferencia Internacional sobre las Neumoconiosis | *Parte* **I**



Pittsburgh, Pennsylvania, USA—August 23–26, 1988
Pittsburgh, Pennsylvanie, Etats-Unis—23–26 aout 1988
Pittsburgh, Pennsylvania EE. UU—23–26 de agosto de 1988

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health

CDC
CENTERS FOR DISEASE CONTROL

Sponsors

International Labour Office (ILO)
National Institute for Occupational Safety and Health (NIOSH)
Mine Safety and Health Administration (MSHA)
Occupational Safety and Health Administration (OSHA)
Bureau of Mines (BOM)

September 1990

DISCLAIMER

Sponsorship of this conference and these proceedings by the sponsoring organizations does not constitute endorsement of the views expressed or recommendation for the use of any commercial product, commodity, or service mentioned.

The opinions and conclusions expressed herein are those of the authors and not the sponsoring organizations.

DHHS (NIOSH) Publication No. 90-108 Part I