Analysis of chromosomal aberrations in shoe workers exposed long term to benzene

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Abstract

Cytogenetic analysis of peripheral blood lymphocytes was used to compare 58 shoe workers (57 men and one woman) exposed to benzene and 20 subjects selected from the general population not exposed to particular mutagenic or carcinogenic agents (control group). Frequencies of damaged cells, including gaps, breaks, and rearrangements (acentric fragment, deletion, translocation) were scored for both groups. The incidence of chromosomal aberrations (particularly chromatid gaps and breaks) in the exposed group was significantly higher than in the control group. There were no effects of smoking and only breaks were affected by alcohol. Nor was there a significant relation between the working period in the group exposed to benzene and frequency of chromosomal aberrations.

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Benzene and its derivates are important substances widely used in industry. Many workers are professionally exposed to the agents for a long time. Chronic benzene intoxication modulates immune responses and granulocyte enzyme systems, and leads to thrombocytopenia, leukopenia, anaemia, and pancytopenia; in extreme cases it can give rise to neoplastic diseases.[1–5] The frequency of neoplasia in workers professionally exposed to benzene and its derivatives is significantly higher than in the unexposed population.[6–8] Sasiadek *et al* showed that numerical or structural chromosomal aberrations were increased in workers exposed long term to benzene.[9] Furthermore, they found that the chromosomal aberration ratios were increased by up to 55% in leukaemia that occurred after exposure to benzene. Sarto *et al* noted slight increases in chromosomal aberrations in lymphocyte cultures from 12 healthy workers in factories producing benzene, toluene, and xylene. These authors believed that this may have resulted from either recent exposure to low concentrations of benzene or to past exposures.[10] Clare *et al* made a cytogenetic evaluation in peripheral blood lymphocyte cultures from 10 workers exposed to high concentrations of benzene as a result of a spillage of about 1200 gallons during the loading of a ship three months earlier. When they compared these chromosomal analyses with those from 11 normal men they found no significant differences between the two groups.[11] Yardley-Jones *et al* found in their study made on 48 workers exposed to low concentration of benzene and 29 controls that chromosomal aberrations (particularly chromatid deletions and gaps) were slightly higher in the exposed group.[12] Jablonicka *et al*, however, showed in their study on 66 workers exposed to benzene that although the frequency of chromosomal aberrations, including breaks and chromosomal exchanges, was slightly increased in the exposed group it was not significant.[13] At the same time, they showed that benzene caused no health problem in these workers according to biochemical and haematological tests.

In our study, the chromosomal aberrations in shoe workers exposed to benzene and its derivatives because of their occupation were evaluated.

Materials and methods

The studies were carried out on peripheral blood lymphocytes of 58 shoe workers occupationally exposed to benzene and its derivatives for five to 50 years.

Twenty healthy subjects who were not exposed to benzene or any physical or chemical agents and living in the same area were taken as a control group. Both groups were interviewed about infectious diseases, drugs, and exposure to x rays during the past two to three months before cytogenetic examination. It was found that 68% of shoe workers and 35% of the control group were smokers, and 39% of shoe workers and 5% of the control group drank alcohol. Blood samples were taken between May 1991 and July 1992. They were cultured at 37°C for 72 hours in medium containing 10% TC medium 199, 20% fetal calf serum, 10 μg/ml phytohaemagglutinin (PHAM), 100 μg/ml streptomycin, 60 μg/ml penicillin, and 70% sterilised distilled water. Colchicine was added to the cultures two hours before the harvest. Four chromosomal preparations were made from each subject according to a routine method.[14] About 20 metaphases were analysed for numerical and structural aberrations under 1000 × magnification with immersion oil. In the control group 435 metaphases and in shoe workers 1079 metaphases were worthy of examination.

STATISTICAL ANALYSIS

Chromosomal findings were compared between groups with the Duncan test. The

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working period and alcohol intake of the shoe workers and smoking habits of both groups were compared with chromosomal findings by regression analysis and Student's t tests.

Results

Tables 1 and 2 show the results of cytogenetic analysis in non-exposed controls and benzene exposed shoe workers. Statistical analysis (including and excluding gaps) showed significant differences in frequencies of chromosomal aberrations between exposed and non-exposed groups (table 3). In the control group the mean frequency of cells with total chromosomal aberrations was 3·35%. In the shoe workers, the frequency was 22·2%. Frequent gaps and breaks (particularly chromatid gaps and breaks) were found among total chromosomal abnormalities (15·7% and 4·3%). Rearrangements such as acentric fragments, deletions, and translocations were found less often (0·6%). These abnormalities were not found in the control group. Only polyploidy was found as a numerical chromosomal abnormality (1·8%) in the workers.

Table 4 shows the comparison of chromosomal findings with working period, smoking, and alcohol intake. No significant differences were found except for a relation between alcohol intake in shoe workers with breaks.

Discussion

In our study, workers occupationally exposed to benzene and its derivatives long term showed a significantly increased frequency of metaphase cells with structural chromosomal aberrations (including or excluding gaps) over that found in a control population. The most often observed aberrations were gaps and breaks. Generally gaps and breaks were the chromatid type. The rearrangement ratios such as acentric fragments, deletions and translocations were significantly increased in workers *v* the control group. Acentric fragments were the most frequent rearrangements: deletions and translocations were rare. Although numerical abnormalities were increased slightly in workers *v* the control group the means did not differ significantly.

Previous studies on workers who have been exposed to benzene reported that chromosomal abnormalities were increased[2 9 11] and our study also seems convincing. Jablonicka *et al*, however, showed that frequency of chromosomal aberrations was not significantly increased in workers exposed to benzene long term when compared with a control group.[13] The difference may be due to the concentration of benzene to which the workers in our study were exposed. When we compared all exposed workers and controls according to smoking and alcohol intake we found no significant differences in total numerical and structural chromosomal aberrations (table 4, $p > 0·05$). Also, we did not find any significant relation between the frequency of chromosomal aberrations and the working period.

Other investigators could not find any connection between chromosomal aberrations, frequency, age, sex, and working period, even though the frequency was higher in workers exposed to benzene than in control groups.[2 11 15] Yardley-Jones *et al*, however, found that chromosomal exchanges and the other chromosomal aberrations were increased in their study in older workers who were exposed to benzene long term.[12] Aksoy *et al* found the appearance of different types of neoplasia as a result of long term exposure to benzene.[2] Furthermore, Sasiadek *et al* showed that there was an increase in abnormal chromosomal aberrations in leukaemia that occurred in workers exposed to benzene.[9]

There is a relation between many malignancies and chromosomal abnormalities.[16] Chromosomal damage has an important role in the activation of proto-oncogenes and their interaction plays an indirect part in the formation of malignancy.[16] Although the metabolism and toxicity of benzene in the organism are known, the mechanism of neoplastic cell transformation induced by benzene has not yet been elucidated. Activation of the tyrosine kinase group of oncogenes may play a key

Table 1 Percentage of cells with chromosomal damage in peripheral blood lymphocyte cultures from control subjects

Subject	Age (y)	Alcohol intake	Daily smoking (pack)	No of metaphases analysed	Gaps	Breaks	Rearrangements	Polyploidy	Total +gaps	Total −gaps
1	24	−	0	20	0·00	0·00	0·00	0·00	0·00	0·00
2	31	−	0	30	0·00	0·00	0·00	0·00	0·00	0·00
3	23	−	0	20	0·00	0·00	0·00	0·00	0·00	0·00
4	26	+	1	30	0·00	0·00	0·00	0·00	0·00	0·00
5	28	−	0	20	5	0·00	0·00	0·00	5	0·00
6	31	−	0·5	20	0·00	0·00	0·00	0·00	0·00	0·00
7	23	−	0	25	0·00	0·00	0·00	0·00	0·00	0·00
8	24	−	0	20	5	0·00	0·00	0·00	5	0·00
9	28	−	0	20	0·00	0·00	0·00	0·00	0·00	0·00
10	24	−	1	20	5	0·00	0·00	0·00	0·00	0·00
11	27	−	0	20	0·00	0·00	0·00	0·00	0·00	0·00
12	29	−	0	25	8	0·00	0·00	0·00	8	0·00
13	29	−	0·5	20	0·00	0·00	0·00	0·00	0·00	0·00
14	23	−	0·1	20	10	5	0·00	5	20	10
15	36	−	0·33	20	5	0·00	0·00	0·00	5	0·00
16	36	−	0·75	25	4	0·00	0·00	0·00	4	0·00
17	32	−	0	20	5	0·00	0·00	5	10	5
18	31	−	0	20	0·00	0·00	0·00	0·00	0·00	0·00
19	35	−	0	20	0·00	0·00	0·00	0·00	0·00	0·00
20	29	−	0	20	5	0·00	0·00	0·00	5	0·00
Mean(SD)	28·5 (4·8)			21·8 (3·4)	2·6 (3·2)	0·25 (0·1)	0 (0·0)	0·5 (1·5)	3·4 (5)	0·75 (2·4)

Table 2 Percentage of cells with chromosomal damage in peripheral blood lymphocyte cultures from benzene exposed shoe workers

Subject	Age (y)	Period of exposure (y)	Alcohol intake	Daily smoking (packet)	No of metaphases analysed	Gaps	Breaks	Rearrangements	Polyploidy	Total +gaps	Total −gaps
1	26	16	−	1	18	11·11	11·11	0·00	11·11	33·33	22·22
2	37	29	−	2	17	17·64	0·00	0·00	0·00	17·64	0·00
3	44	35	−	0·5	17	29·41	0·00	0·00	5·88	35·29	5·88
4	24	12	+	1	17	17·64	0·00	0·00	0·00	17·64	0·00
5(F)	43	11	−	0	10	20	0·00	0·00	0·00	20	0·00
6	41	29	−	1	17	11·76	11·76	5·88	0·00	29·41	17·65
7	41	28	−	1	19	21·05	0·00	0·00	0·00	21·05	0·00
8	29	19	−	1	23	13·04	8·69	4·34	0·00	26·08	13·04
9	40	30	−	0	13	23·07	0·00	0·00	0·00	23·07	0·00
10	34	21	−	0	13	15·38	0·00	0·00	0·00	15·38	0·00
11	54	44	+	0	15	20	0·00	0·00	0·00	20	0·00
12	43	28	−	1	25	12	4	0·00	0·00	16	4
13	52	30	−	0	20	10	0·00	0·00	0·00	10	0·00
14	39	20	−	0	17	11·76	0·00	5·88	0·00	17·64	5·88
15	22	11	+	1	37	13·51	2·70	0·00	0·00	16·21	2·70
16	33	20	+	1	13	0·00	7·69	0·00	0·00	7·69	7·69
17	24	10	+	0·5	43	18·60	9·30	0·00	4·65	32·55	13·95
18	47	37	−	1	10	20	0·00	0·00	0·00	20	0·00
19	60	50	+	0	10	10	20	0·00	0·00	30	20
20	24	11	+	1	22	9·09	9·09	0·00	0·00	18·18	9·09
21	32	20	+	2	14	0·00	14·28	0·00	7·14	21·42	21·42
22	24	12	−	0	9	11·11	0·00	0·00	22·22	33·33	22·22
23	44	35	+	0	23	8·69	4·34	0·00	0·00	13·04	4·34
24	34	23	+	0	19	10·52	10·52	0·00	5·26	26·31	15·79
25	42	25	−	0·3	17	11·76	5·88	5·88	5·88	29·41	17·65
26	38	11	+	1	17	11·76	11·76	0·00	0·00	23·52	11·76
27	33	24	+	1	21	9·52	9·52	0·00	9·52	28·57	19·05
28	30	17	+	1·5	15	13·33	6·66	0·00	0·00	20	6·66
29	69	50	−	0	30	13·33	6·66	3·33	0·00	23·33	10
30	31	22	+	1·5	15	6·66	6·66	0·00	0·00	13·33	6·66
31	31	12	+	2	20	10	10	0·00	0·00	20	10
32	42	31	+	1·3	20	10	0·00	0·00	0·00	10	0·00
33	22	8	+	0	17	11·76	5·88	0·00	0·00	17·64	5·88
34	22	11	+	1	26	15·38	3·84	0·00	0·00	19·23	3·84
35	43	30	−	0	8	12·50	0·00	12·50	0·00	25	12·5
36	27	14	+	0·5	13	7·69	0·00	0·00	7·69	15·38	7·69
37	42	22	+	1·7	12	8·33	8·33	0·00	8·33	25	16·67
38	46	38	−	0·5	11	18·18	9·09	0·00	0·00	27·27	9·09
39	48	38	+	1·7	21	14·28	4·76	4·76	4·76	28·57	14·29
40	27	7	+	0	10	20	0·00	0·00	0·00	20	0·00
41	27	10	−	1	24	8·33	4·16	0·00	0·00	12·5	4·16
42	28	13	−	0	20	20	10	0·00	0·00	30	10
43	47	35	−	0·8	15	13·33	0·00	6·66	0·00	20	6·66
44	46	32	−	3	18	5·55	5·55	0·00	0·00	11·11	5·55
45	39	25	−	1	26	19·23	0·00	0·00	3·84	23·07	3·84
46	23	10	−	1	13	15·38	7·69	7·69	0·00	30·76	15·38
47	24	5	−	1	11	27·27	0·00	0·00	0·00	27·27	0·00
48	46	30	−	1	21	19·04	0·00	0·00	0·00	19·04	0·00
49	45	30	−	1·5	15	33·33	0·00	0·00	0·00	33·33	0·00
50	41	30	−	1	29	24·13	0·00	3·44	3·44	31·03	6·9
51	24	8	−	0	30	16·66	0·00	0·00	0·00	16·66	0·00
52	25	8	−	0	10	30	0·00	0·00	0·00	30	0·00
53	61	40	−	1	20	25	0·00	0·00	0·00	25	0·00
54	39	25	−	2	26	19·23	3·84	0·00	0·00	23·07	3·84
55	24	10	−	1	21	23·80	0·00	0·00	0·00	23·80	0·00
56	45	30	−	0·8	30	13·33	0·00	3·33	0·00	16·66	3·33
57	50	35	+	1·5	25	20	0·00	0·00	4	24	4
58	40	30	−	0	11	9·09	9·09	0·00	0·00	18·18	9·09
Mean(SD)	37·2±10·9	23·2±11·4		0·8±0·7	18·6±7·1	15±6·8	4·2±4·8	1·39±3·3	1·8±3·9	22·1±6·7	7·08±6·84

part in the neoplastic transformation of cells.[5 17 18] Benzene may affect the tyrosine kinase groups of oncogenes by causing chromosomal damage and as a result of this malignancy may arise. No direct relation could be found, however, in the previous studies or in our study, between the increase of chromosomal abnormalities and cancer in workers exposed to benzene long term. Although chromosomal abnormalities were high in our study, no symptom described in the interview related to malignancy, symptoms tended to relate to stomach, skin, and respiratory complaints. This situation would suggest that the development of cancer is a multistep process and the increase in chromosomal abnormalities only is insufficient in this development. We think that the reason for the high frequency of chromosomal abnormalities in our study may be because of high concentrations of benzene in the working environment.

In Turkey, although the acceptable maximum allowable concentration (MAC) in the working environment is 20 ppm, Aksoy pointed out that the maximum benzene value was between 210 to 610 ppm during working hours in workplaces in Istanbul.[19] This may be because of the content of benzene (more than 1%) and hexane (more than 44%) in adhesives. Also, unhealthy working conditions such as narrow working places with insufficient ventilation and a lack of hygiene could be other factors. In our country, moves are being made to limit the content of hexane to 55 ppm and to decrease the MAC from 20 ppm to 1 ppm.

In conclusion, chromosomal abnormalities are increased in the workers exposed to benzene long-term in our study. It is recommended that the MAC concentration should be decreased from 20 ppm to 1 ppm and working conditions should be improved.

Table 3 Comparison of chromosomal data of shoe workers and controls

Compared criteria	*Means*		$Ft_{(a = 0.05)}$	F_h	*p Value*
	Shoe workers (n = 58)	*Controls (n = 20)*			
Break	4·187	0·25	3·13	12·94	**<0·05
Gap	15·044	2·6	3·13	61·51	<0·05
Rearrangements	1·399	0	3·13	3·69	<0·05
Polyploidy	1·788	0·5	3·13	2·016	NS
Total:					
+ gap	22·121	3·35	3·13	130·4	<0·05
− gap	7·075	0·75	3·13	16·255	<0·05

Table 4 Comparison of chromosomal findings with working period and alcohol intake of shoe workers and smoking habits of both groups by regression analysis and Student's t *test*

Compared criteria I with	*II*	*Correlation coefficient (r)*	t_h	*p Value*
Working period	Break	0·006	0·006	NS
	Gap	0	0	NS
	Rearrangement	0·0389	0·0943	NS
	Polyploidy	0	0	NS
	Total	0	0	NS
Alcohol intake	Break	0·263	2·04	**<0·05
	Gap	−0·405	−3·33	NS
	Rearrangement	−0·1107	−0·834	NS
	Polyploidy	0·0638	0·478	NS
	Total	0	0	NS
Smoking	Break	0·149	1·1298	NS
	Gap	−0·168	−1·281	NS
	Rearrangement	−0·067	−0·502	NS
	Polyploidy	−0	−0·185	
	Total	0	0	NS

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