

Benzene-induced Chromosome Aberrations: A Follow-up Study

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- ° [Abstract](#)
- ° [Introduction](#)
- ° [Methods](#)
- ° [Results](#)
- ° [Discussion](#)

Abstract

To study the evolution of cytogenetic damage from past exposure to high concentrations of benzene and its health significance, chromosome aberrations (CA) in lymphocytes were reinvestigated after approximately 20 years in four subjects with past severe hemopathy and in seven controls studied in the late 1960s. Increased chromosome-type aberrations were still present up to 30 years after benzene toxicity, but blood counts were normal. The vital status at the end of 1993 was ascertained for 32 subjects with a history of benzene toxicity and for 31 controls studied for CA from 1965 to 1970, who differed significantly for CA rates. Of the 32 benzene-exposed subjects, 1 was lost to follow-up, 20 were still alive, and 11 had died at ages 36 to 83, between 1 and 20 years after the last CA study. Five deaths were from neoplasia (acute erythroleukemia, brain tumor, cancer of lung, paranasal cavity, esophagus). The deceased subjects had significantly higher rates of chromosome-type aberrations than those alive, and those who died of neoplasia had the highest rates of these aberrations in the last study before death or diagnosis of cancer. Out of the 31 controls, 12 had died from 4 to 23 years after the CA study. Three deaths were from neoplasia (two lung cancer, one brain tumor). Even if this is a small sample, the results suggest a higher **risk** of cancer for the benzene-exposed cohort, who had persistently high CA rates in lymphocytes. -- Environ Health Perspect 104(Suppl 6): 1309-1312 (1996)

Key words: benzene toxicity, chromosome aberrations, genotoxic effects, lymphocytes

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Abbreviations used: CA, chromosome aberrations; ct-A, chromatid-type aberrations; cs-A, chromosome-type aberrations; C_u cells, cells with unstable chromosome-type aberrations; C_s cells, cells with stable chromosome-type aberrations.

Introduction

When inhaled at high concentrations, benzene is myelotoxic and leukemogenic for humans, and it is known to induce structural and numerical changes in experimental animals and in man (1). Chromosome aberrations (CA) in cultured peripheral blood lymphocytes are a biomarker of early biological effect of exposure to genotoxic agents in humans (2). In the late 1960s, CA in peripheral blood lymphocytes were repeatedly studied in 32 subjects with a history of occupational benzene poisoning and were significantly increased in most exposed individuals, compared to matched controls, even several years after cessation of exposure and recovery from toxicity (3).

The prognostic significance of increased CA in lymphocytes, especially if persistent in time, for later cancer **risk**, is still debated. It is generally agreed that CA might be indicators of increased cancer **risk** at a group level but not at the individual level (2). Long-term follow-up of individuals and groups investigated for CA is necessary to solve this problem.

With this purpose in mind, stimulated by the work of Saracci (4), a follow-up study was started in the 1980s of benzene-exposed cases cytogenetically studied in the 1960s (3) and of a group of controls investigated in the same period. In particular, CA in lymphocytes were studied again in a few exposed cases and controls after approximately 20 years, and the vital status of cases and controls was ascertained and the cause of death was registered. The results of this updated follow-up study are reported here.

Methods

The 32 cases followed up (17 males, 15 females) are those first studied from 1965 to 1970, reported by Forni et al. (3); i.e., 25 subjects who had suffered from benzene hemopathy (severe in 15 cases) 1 to 18 years before the first CA study; 4 subjects with signs of benzene-induced bone marrow toxicity at time of the first cytogenetic study; and 3 subjects recovered from acute benzene poisoning. Case numbers, when reported, are those used for identification in the original study (3).

Out of the 31 referents (22 males, 9 females), 14 were matched controls in the original study, and the others were healthy subjects cytogenetically studied in the same period who served as controls for other studies.

Four female cases (nos. 15, 16, 24, and 26), who had suffered from severe benzene hemopathy in 1961, 1962, 1958, and 1968, respectively, and had received several CA studies in the sixties, were restudied from 1985 to 1988; case no. 26 received one additional cytogenetic study in April 1995. Seven referents (5 males and 2 females), first studied from 1965 to 1969, were reinvestigated from 1986 to 1991. Complete blood counts and updating of clinical histories were performed at the time of cytogenetic follow-up. Informed consent for cytogenetic reinvestigation was obtained.

The vital status of the 32 cases and the 31 controls was first ascertained through the municipality of residence by T. Vai of our institute, and again in 1993 as a part of a multicenter investigation (5). The cause of death was drawn from the death certificates, but for some cases and controls the clinical history and more precise hospital diagnoses were reported.

Lymphocyte cultures in the 1960s were performed using two methods and harvested at 68 to 70 hr (3). The cytogenetic reinvestigations in the 1980s were carried out on 48- and 70-hr cultures of whole blood with no significant differences, but the results of 3-day cultures were used for a better comparison with the old data. One hundred metaphases were counted and scored for structural CA according to the International System for Human Cytogenetic Nomenclature (ISCN) (6). For the old data, the original protocols were reviewed, and chromatid-type aberrations (ct-A) (excluding gaps), which in the original study had been scored but not reported, were included in the evaluation. Hyperdiploid cells with apparently normal extra chromosomes were separated from cells with stable aberrations and recorded as such. Aneuploidy was evaluated only considering percentage of hyperdiploid cells, since hypodiploid

cells in lymphocyte cultures may result from artifactual loss.

For statistical analysis, cells with structural aberrations were grouped as: ct-A, with chromatid-type aberrations; C_u , with unstable chromosome-type aberrations (acentric fragments, dicentric and ring chromosomes); and C_s , with stable chromosome aberrations (abnormal monocentric chromosomes due to deletions, translocations, inversions).

Statistical comparison, when required, was carried out by the Mann-Whitney U-test.

Results

Cytogenetic and Clinical Follow-up

At the time of reinvestigation, both cases and controls had normal blood counts and were clinically healthy, with the exception of case no. 24, who had type II diabetes and had been under treatment with oral antidiabetics for the last 10 years.

The results of the first and last CA studies in four cases and seven controls are summarized in Table 1. The rate of chromatid-type aberrations did not differ between cases and controls. CA were significantly higher in the exposed subjects than in the controls in the first study; in the last study, they were still higher, but the difference was not statistically significant due to an increase of C_u in three out of seven controls. The differences between cases and controls were more pronounced when considering cells with complex aberrations involving more than one break (data not shown).

Table 1. Chromosome aberration rates (mean values) in four females who suffered from severe benzene hemopathy in 1958 to 1968 [exposed] and in seven controls (two females, five males) (control).

		Age mean	ct-A	% Cells C_u	C_s	% Total abnormal metaphases	% Hyperdiploid cells
First study ^a	Exposed	27	1.0	1.5*	1.0 [†]	3.7	1.0 [†]
	Control	36	1.6	0.4*	0**	2.0	0 [†]
Last study ^b	Exposed	50	3.7	2.7	0.3	6.7	1.3 [†]
	Control	56	1.3	1.4	0	2.7	0 [†]

^aIn 1965–1968. ^bIn 1986–1995. Mann-Whitney U-test: * $0.055 > p > 0.036$; ** $0.036 > p > 0.021$; [†] $0.036 > p > 0.021$; [‡] $0.036 > p > 0.021$.

It is interesting that C_s in the exposed subjects have decreased over the last two decades, while there has been an increase of C_u , which paralleled that of the controls. Hyperdiploid cells were significantly increased in the exposed subjects versus the controls both in the first and in the last study (Table 1).

Case no. 26, studied for the first time when severe pancytopenia developed in late pregnancy, should be noted. The long-term follow-up shows an overall decrease in structural CA (Table 2). In the most recent study, this woman, now 49 years old, had one dicentric chromosome and three hyperdiploid cells in 100 metaphases. Blood counts were normal. She had no complaints. Recently, in a check-up performed because she wanted to become a volunteer blood donor, she has been found positive for HBV and HCV antibodies but negative for HBV antigens. Her 27-year-old healthy son, who was born in 1968 when the patient received numerous blood transfusions, is also positive for HBV and HCV antibodies. This woman, after benzene poisoning, delivered two more babies (one male, one female) both normal and currently healthy.

Table 2 Cytogenetic follow-up of a case of severe chronic benzene poisoning (case no. 26, female, 22 years old in 1968): mean results of several studies.

n	CA studies Years	ct-A	% Cells C _u	C _s	% Total abnormal metaphases	% Hyperdiploid cells
5	1968–1969 ^a	2.0	2.2	2.8	7.0	1.4
3	1969–1974	3.0	2.3	0.3	5.6	0
2	1980–1988	1.0	2.0	1.5	4.5	0
1	1995	0	1.0 ^b	0	1.0	3.0

Abbreviations: ct-A, cells with chromatid-type aberrations; C_u, cells with unstable chromosome-type aberrations; C_s, cells with stable chromosome-type aberrations. ^aAt time of pancytopenia. ^bDicentric chromosome.

The pregnancy outcomes of the two other married cases are interesting as well. Case no. 15 had one normal pregnancy in 1971 and delivered one normal daughter. Case 16 had five normal pregnancies in 1966, 1967, 1969, 1970, and 1975, respectively, and delivered four males and one female; the first baby died at 7 months of bronchopneumonia, the others are healthy.

Vital Status and Clinical Data

The results of the ascertainment of vital status for benzene-exposed subjects and controls are summarized in Table 3.

Table 3 Vital status of 32 cases^a with histories of benzene poisoning and 31 controls.

	n	Age, years, mean (range)
Cases (17 M, 14 F)		
Dead	11	59.8 (36–83)
Neoplasia	5	54.8 (36–69)
Other causes	6	64.0 (49–83)
Alive	20	59.7 (44–74)
Controls (22 M, 9 F)		
Dead	12	60.2 (23–85)
Neoplasia	3	69.0 (55–79)
Other causes	9	57.3 (23–85)
Alive	19	59.4 (46–79)

Abbreviations: M, male; F, female. ^aOne case lost to follow-up.

Out of 32 cases, 1 was lost to follow-up; 20 are alive; and 11 died 1 to 20 years after the last cytogenetic study. Of these, five died of neoplastic disease (case no. 9, age 67, erythroleukemia; case no. 18, age 43, cancer of the paranasal cavity; case no. 19, age 36, brain tumor; case no. 20, age 59, lung cancer; case no. 24, age 69, cancer of the esophagus). The cancer deaths occurred 10 to 34 years after benzene poisoning, 2 to 27 years after the first CA study, and 1 to 4 years after the last cytogenetic study. Four of the five cancer deaths are clustered between 2 and 6 years from the first CA study. Four of the 5 cancer deaths and 3 deaths from nonneoplastic disease occurred among the 15 cases who had suffered from severe benzene hemopathy. A few cases deserve a special comment.

Case no. 9, who died in 1973, had suffered from severe benzene hemopathy in 1957 at age 51 and had completely normal blood counts in 1967 to 1969 when she participated in three CA studies, which showed, respectively, 4, 4, and 5% CA (with complex aberrations). In 1972, she developed pancytopenia with immature cells of the white and red blood series in the peripheral blood. A CA study of cultured blood lymphocytes in September 1972 showed 4% C_u (including one dicentric and two ring chromosomes). Three bone marrow punctures were void. One direct preparation of peripheral blood

yielded two metaphases only, not suitable for analysis. The final diagnosis was acute erythroleukemia. This was the last case of benzene leukemia observed in our institute and was, in our experience, the only case of late onset leukemia, with a latency time of 15 years from cessation of exposure to benzene.

Case no. 18, who died from cancer of the left maxillary sinus at the age of 43, had suffered from severe benzene hemopathy at the age of 32 and developed cancer of the paranasal cavity at the age of 40. He had been a shoemaker since age 16, in different factories, engaged in various jobs involving exposure to both benzene-containing glues and leather dust.

Case no. 20, who died from lung cancer at age 59 in 1969, was a smoker. He had worked in a rotogravure plant and had suffered from severe benzene poisoning in 1953.

Case no. 31, who suffered from acute benzene poisoning of moderate degree and died at age 83 of unspecified heart disease, had undergone surgery for breast cancer in 1969, as already reported (3).

Among the 31 controls, 19 are alive and 12 died 4 to 23 years after the unique CA study (Table 3). Three died of neoplastic disease (one male, age 55, smoker, lung cancer; one male, age 73, brain tumor; one female, age 79, nonsmoker, lung adenocarcinoma) 13, 14, and 19 years after the CA study, respectively. Among the controls were two deaths from car accidents (one female, age 23; one male, age 50).

As shown in Table 3, the overall age at death of cases and controls is similar. However, among the cases the cancer deaths occurred at younger ages (mean 54.8 vs 69.0), but the difference did not reach statistical significance.

Cytogenetic Findings and Vital Status

To answer the question of the prognostic significance for cancer risk of increased CA in lymphocytes, the results of chromosome aberration studies of cases and controls were compared according to vital status (Table 4). When, as for most exposed subjects, more than one CA study had been performed, the latter was used. For those who died of neoplastic disease, the last cytogenetic study before the diagnosis of cancer was taken into account.

Table 4 Chromosome aberration rates in lymphocytes (mean values) in the last or unique cytogenetic study of 31 benzene cases and 31 controls according to vital status.

	<i>n</i>	% Cells with ct-A	% Cells with cs-Δ	% Total abnormal metaphases
Cases (17 M, 14 F)				
Dead	11	2.6	3.9*	6.5
Neoplasia	5	3.4	4.4	7.8
Other causes	6	2.0	3.5	5.5
Alive	20	3.6	2.3*	5.9
Controls (22 M, 9 F)				
Dead	12	1.5	1.0	2.5
Neoplasia	3	2.3	0.7	3.0
Other causes	9	1.2	1.1	2.3
Alive	19	0.6	1.0	1.6

*Mann-Whitney U-test: $0.025 > p > 0.01$.

The CA rates of cases and controls, either dead or alive, were significantly different both for cells with chromosome-type aberrations and for total abnormal metaphases ($p < 0.001$) (statistical significance not shown in the Table 4).

When considering the cases, those who died showed significantly higher rates of CA but not of total abnormal metaphases than those who are still alive. Those who died of cancer presented the highest

values. No such differences, however, were present among the controls (Table 4). Also, no differences were present for hyperdiploid cells (data not shown).

Discussion

To the best of my knowledge, data on long-term cytogenetic follow-up of subjects exposed in the past to high concentrations of a clastogenic-leukemogenic agent such as benzene are scanty. The only data are those of Pollini and Biscaldi (7), who described a return to normal of rates of aneuploid cells (mainly hypodiploid) in four subjects who had been first studied 12 years earlier, at the time of benzene hemopathy.

In our experience, however, increased rates, as compared to controls, of chromosomal abnormalities (mainly structural) were still present in most cases up to 14 to 18 years after chronic benzene poisoning, and the findings have been confirmed in serial examinations (3).

The present data concerning cytogenetic reexamination of cultured lymphocytes approximately 20 years later in four subjects recovered from benzene hemopathy again show the persistence of increased C. However, an increase in this type of aberration compared to the earlier findings was also present among the controls. This age effect, which seems common to cases and controls, might possibly represent cumulative chromosome damage from exposure to genotoxic agents or might depend on a reduced capacity of repair (8). It might be interesting to evaluate whether this increase correlates with the well-known increased risk for cancer with aging in a population.

From the review of the old data plus the new data of this follow-up, it seems that, at least for benzene exposure, the rates of CA are a better indicator of the *in vivo* situation than those of total abnormal metaphases, as is the case for exposure to ionizing radiation (8).

As far as the prognostic significance of increased CA for cancer risk is concerned, the follow-up data of the cohort of benzene-exposed subjects and controls, who differed significantly for CA rates, seem to indicate a trend toward a higher risk of neoplasia and a younger age at death from cancer for subjects recovered from benzene hemopathy, even though the small numbers do not allow a definitive conclusion. Among the exposed, those who died from neoplasia were also the ones who had the highest rates of CA in the last cytogenetic study before the diagnosis of cancer.

Two large, ongoing epidemiological studies, one from Northern Europe (9) and one from Italy (5), seem to indicate an approximately double risk of cancer incidence or death, respectively, for the subgroups of subjects with high, compared to those with low, CA levels. The results of the present study, despite the small sample size, seem in agreement with these conclusions.

Two major points, however, should be stressed: a) on one hand, this study concerns a group of subjects with exposure to very high concentrations of benzene, in the order of hundreds of parts per million, which had induced in most cases important signs of toxicity; b) on the other hand, this study does not take into account the early deaths from aplastic anemia or leukemia that were observed at our institute in the 1960s (10).

Further follow-up of this or similar cohorts is highly desirable. Moreover, the application in cytogenetic studies of exposed and controls, of modern, more sensitive techniques that might enable a better evaluation of stable or more subtle chromosomal changes underscored in conventional lymphocyte cultures might help answer some of the open questions.

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