Chromosome Aberration Analysis in Persons Living in the Vicinity of the Nuclear Power Plant Krümmel

Anna Heimers, Bettina Dannheim, Ingeborg Grell-Büchtmann, Heike Schröder, Inge Schmitz-Feuerhake, Bremen

Introduction

Between November 1989 and May 1991 five cases of childhood leukaemia were diagnosed in the community of Elbmarsch, which consists of a chain of small villages situated opposite the boiling water reactor Krümmel (KKK) at the river Elbe. Additionally there was one leukaemia case in a 21 year old young male and an aplastic anaemia in a child. Since 1994 four additional cases appeared. The total number of inhabitants of the community of Elbmarsch is approximately 8800 including 1600 children <15 years of age.

The governments of the Federal States of Lower Saxony and Schleswig-Holstein established a board of experts to identify possible causes for the observed leukaemia cluster. Potential risk factors such as X-rays, chemicals, and previous deseases of the affected families were evaluated by official investigation and excluded. None of the parents had been exposed to occupational or unusual medical irradiation. One family, however, was found to be living in a house with a mean radon concentration of 450 Bq/m^3 in their living rooms. The only common factor detected which could possibly explain the observed leukaemia cluster is its proximity to the nuclear power plant KKK.

To examine whether the Elbmarsch population in the near vicinity of KKK was subjected to elevated exposures of ionising radiation, chromosome aberration analysis of dicentric chromosomes in the lymphocytes of the peripheral blood was carried out on a group of inhabitants.

Material and Methods

The retrospective investigation comprised blood samples from 21 individuals (19 females and 2 males), all of them inhabitants of the Elbmarsch community living within a distance of 5 km in a southern direction from KKK, or former inhabitants of this area. At the time the blood samples were taken (in 1992, 1993 and 1995) these volunteers were aged 28-45 years. Four women were selected because of the fact that they had settled in the village after 1986, as had one of the leukaemia families. Seven subjects were parents of the children diagnosed as having leukaemia in the community of Elbmarsch.

The control group consisted of 25 healthy adult persons (9 females, 16 males) living in the city of Bremen. At the time of blood sampling between 1988 and 1994 they were aged 17-57 years. A detailed questionnaire was completed by each of the subjects. Exclusion criteria were previous occupational exposures, greater than average diagnostic medical irradiation, or exposure to chemical mutagens. Smokers (more than 10 cigarettes per day) were also excluded.

Blood samples were drawn by venipuncture. Lymphocyte cultures and slide preparations were made according to standard cell cycle controlling methods (Fluorescence-plus-Giemsa staining). From each donor, five cultures were established containing 0.5 ml whole blood, 5.6 ml RPMI 1640 medium (Sigma) supplemented with 17% fetal calf serum (Seromed), 500 IU Liquemin (Hoffmann-LaRoche), 0.5 mg streptomycin, 500 IU penicillin (Boehringer, Mannheim), 0.027 mg bromodeoxy-

uridine (BrdU, Sigma), and 0.036 mg phytohaemagglutinin (Seromed).
The cultures were incubated for 48 h at 37°C in the dark. After 45 h Colcemid (Boehringer, Mannheim) was added (0.34 µg/ml). The cells were treated with KCl (0.075M) for 15 min at 37°C and then fixed in methanol:acetic acid (3:1; v:v) at 4°C. Fluorescence-plus-Giemsa technique was performed according to the method of Perry and Wolff [4].
1000 first division metaphases for each case were analysed for every kind of structural aberration. The collection of metaphases was faciliated by a semi-automatic, computerized system which included a data management tool (MetaSystems, Sandhausen, Germany).

Results and Discussion

The results of the chromosome aberration analysis are given in table 1. The rate of dicentric chromosomes in the control group was $(0.46 \pm 0.15) \times 10^{-3}$ per metaphase and was within the range of previous investigations by other authors [1,7,9].
Compared to the control group the investigated population of Elbmarsch showed a highly significant elevation of dicentric chromosomes with a rate of 1.77×10^{-3} per metaphase ($p<0.01$) [3]. The fourfold increase in the rate of dicentrics in inhabitants gives evidence that the population of the Elbmarsch community has been severely exposed to ionising radiation in the past.
Particularly noteworthy was the existence of cells with two dicentrics in the Elbmarsch group (Table 1a; subjects 5,8,10 and 12), in contrast to the control group (no cells with two dicentrics). Additionally there was one metaphase with six dicentrics (Table 1a; subject 3). These aberrations did not follow Poisson distribution but showed a significant overdispersion ($p<0.05$) expressed by the parameter u based on the method by Edwards et al. [2]. This was also valid for the entire Elbmarsch population which was investigated, even if the multiaberrant cell is excluded (Table 2).
Overdispersion of dicentric chromosomes can be caused by either non-uniform or high-LET irradiation. Such distributions have been found in blood samples of persons occupationally exposed to e.g. Plutonium [8], Tritium [5], or Uranium [6]. The observed overdispersion in this study is not to be explained by external gamma-irradiation or other kinds of low dose low LET exposure. A relevant contribution to the population dose by incorporation of α-emitting nuclides, which are not routinely controlled in the environment is assumed.
Dose assessment has not been carried out due to the lacking knowledge about the radiation quality, the exposure pathway, and the time of exposure.

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Table 1
(A Elbmarsch Group; B Control Group)
Results of Chromosome Aberration Analysis in Adult Residents of Elbmarsch and Control

A) Elbmarsch group

No.	parent of leuk. child	resident in Elbmarsch	date of blood sampling	No. of analysed metaphases	No. of dicentrics	rate of dicentrics x 10^{-3}
1	yes	since <1984	Jan. 1992	1005	2	2.0
2	yes	since <1984	Jan. 1992	390	2	5.1
3	yes	since <1984	Jan. 1992	1000	3*	3.0
4	yes	<1984 -1991	Jan. 1992	1001	2	2.0
5	yes	since <1984	Jan. 1992	664	2**	3.0
6	yes	since <1884	Oct. 1995	1010	2	2.0
7	yes	since <1984	Oct. 1995	1010	0	0.0
8	no	since 1988	Apr. 1993	1002	5***	5.0
9	no	since 1987	Apr. 1993	1014	0	0.0
10	no	since 1987	Apr. 1993	1097	4***	3.6
11	no	since 1987	Apr. 1993	1034	1	1.0
12	no	since <1984	Jun. 1993	1005	3***	3.0
13	no	since <1984	Jun. 1993	1110	0	0.0
14	no	since <1984	Jun. 1993	1003	1	1.0
15	no	since <1984	Jun. 1993	1005	1	1.0
16	no	since <1984	Jun. 1993	1002	2	2.0
17	no	since <1984	Jun. 1993	1011	2	2.0
18	no	since <1984	Jun. 1993	1008	0	0.0
19	no	since <1984	Jun. 1993	1007	2	2.0
20	no	since <1984	Jun. 1993	1004	2	2.0
21	no	since <1984	Jun. 1993	1009	0	0.0
Total				20391	36	1.77±0.33§

*) Excluding one multiaberrant cell with 6 dicentrics.
**) One cell contained 1 tricentric which was counted as 2 dicentrics
***) Including one cell with 2 dicentrics.
§) Standard error of the mean

B) Control group

No.	date of blood sample	No. of analysed metaphases	No. of dicentrics	rate of dicentrics x 10^{-3}
1 - 25	1988 - 1995	19775	9	0.46 ± 0.15§

§) Standard error of the mean

Table 2:
Intercellular Distribution of Dicentric Chromosomes in the Investigated Elbmarsch Population

	No. of dicentric chromosomes per cell								
	0	1	2	3	4	5	6	s^2/Y*	u**
incl. multiaberrant cell									
observed	20358	28	4	0	0	0	1	1.90	92.26
expected	20349	41.91	0.04	0	0	0	0		
excl. multiaberrant cell									
observed	20358	28	4	0	0	0	0	1.22	22.58
expected	20354	35.94	0.03	0	0	0	0		

*) relative variance (Y is the mean value)
**) u > 1.96: overdispersion is significant ($p < 0.05$)