

Biodosimetry Study in Dolon and Chekoman Villages in the Vicinity of Semipalatinsk Nuclear Test Site

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Biological dosimetry/Chromosome aberrations/Radiation exposure/Nuclear weapon tests.

In this paper, the results of a biodosimetry investigation are reported for two villages in the area of the Semipalatinsk nuclear testing site: Chekoman and Dolon. Chekoman village is considered to be relatively less affected by radiation in comparison with Dolon village. The distance between the two villages is about 100 km and the life styles of the residents are similar. Chromosome aberrations in lymphocytes collected from the residents of the two villages were analyzed using the fluorescence in situ hybridization technique. Our results showed that the average frequency of stable translocations for the Dolon group was significantly greater than that of the Chekoman group. The elevated level of stable translocations with the Dolon residents corresponds to a dose of about 180 mSv.

INTRODUCTION

In recent years, considerable efforts have been devoted to investigate the consequences of radiation exposures in the area of a nuclear testing site in the Semipalatinsk Region of Kazakhstan. The nuclear weapons tests over a period of 40 years from 1949 to 1989 have been the source of substantial exposure to the residents living in the area, particularly in villages contaminated by the intensive fallout of the radioactive cloud.

Among the villages in the area, Dolon consistently appeared in the literature for its elevated levels of exposure, as the village was directly under the path of the radioactive cloud from the nuclear explosions. From the first test in August, 1949 alone, it was estimated that accumulated external dose might have reached 2 Gy.⁶⁾ Over the entire period of the nuclear testing, the whole body effective dose for Dolon residents was estimated to be 4.47 Sv.^{5,6)}

However, at a meeting in Rome attended by experts in radiological sciences in September, 1997, it was pointed out that a dose of several Gy in some villages based on aerial

surveys to locate the contaminated areas and estimated by modeling is likely to be over-estimated by one or two orders of magnitude. In the World Health Organization report¹²⁾ it was stated that,

“Accumulated doses of the order of 0.1 Gy or less are consistent with the results of biological dosimetry by two independent measurement methods. Stable translocations - which persisted over decades - were studied with Dolon residents but were not found to be elevated as compared to controls from areas not subjected to exposure. These results are a little uncertain as some samples were lost while being transported and only a relatively small number of cells were scored.”

In the same report, it is recommended that “the doses for the inhabitants of Dolon be more concisely estimated and that the basis for previous dose estimates be further investigated”.

In this paper, the results of a biodosimetry investigation are reported for two villages in the area of the Semipalatinsk nuclear testing site, Dolon and Chekoman.

MATERIALS AND METHODS

Subject selection criteria

A total of 15 women were selected from Dolon and 15 women from Chekoman. The women were all born between 1945 and August 1949 and were younger than 5 years old at the time of the first nuclear explosion in August, 1949. The subjects' medical records were reviewed and anyone with a family history of serious disease was excluded. All the subjects had been living in the villages since birth, did not

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smoke and did not consume alcohol heavily. None of the subjects was seriously sick at least within six months before the blood was collected and none of them ever had medical X-rays taken.

Sample preparation

In the village, a total of 10 ml of blood was drawn from each woman into a Vacutainer tube containing Sodium Heparin (100 U.S.P. units) and brought to the Research Institute of Radiation Medicine and Ecology in Semipalatinsk. The 10 ml of blood was then divided into 10 tubes. To each tube containing 1 ml of blood 9 ml of RPMI medium supplemented with 20% calf serum and 1% phytohemagglutinin were added, and the sample was incubated at 37°C. After 48 hours of incubation, colcemid (0.2 µg/ml) was added to the cultures for an additional 4 hour incubation. The cells were then swollen in 0.075 M KCl solution at room temperature for 20 minutes. Samples were fixed in methanol/acetic acid (3:1 vol/vol) fixative solution.

Fluorescence In Situ Hybridization (FISH)

Cells were dropped onto clean slides and aged on a slide warmer for 5 hours at 37°C prior to hybridization. After the spreads were denatured in 70% formamide for 2 minutes at 72°C, they were immediately dehydrated in cold 70%, 85% and 100% ethanol for 2 minutes each, air-dried and placed on a slide warmer at 45°C. A combination of whole chromosome probes (ONCOR) specific for chromosome #1 (orange) and #2 (green) were denatured for 10 minutes at 72°C before being applied to the slide. Spreads were sealed under a cover slip and incubated for 24 h at 37°C in an airtight box containing moist blotting paper.

Slides have been washed at 37°C in two changes of 50%

formamide for 5 minutes each in 2 × SSC for 5 minutes and once in 0.1% NP40 in 2 × SSC for further 5 minutes. After air-drying, 15 ml DAPI were applied as a counterstain. Hybridized spreads were viewed with a Zeiss Axioplan fluorescence microscope.

Classification of chromosome aberrations

For the women exposed decades ago, most aberrations in the lymphocytes are stable translocations. In the present scoring, translocations include both apparently complete translocations and incomplete translocations, as incomplete exchanges are most likely mis-scored completes.¹³⁾ An aberration involving insertion and translocation of the painted chromosome is also scored as one translocation. Apparent clones, as well as direct translocations between chromosomes #1 and #2 were excluded. In the literature, in some classifications^{2,10)} a separate group of “incomplete exchanges” is included. Such a classification is excluded in this work, and such aberrations are scored as one translocation event.

RESULTS

The results of the chromosomal analysis are shown in Fig. 1. As samples from some subjects did not yield enough chromosome spreads for analysis, chromosome aberrations in 10 subjects of Dolon and 5 subjects from Chekoman were analyzed. On some slides, the signal for chromosome #1 was too weak to score and so only the aberrations in chromosome #2 were scored. The number of aberrations in the painted chromosome, F_p , was converted to the equivalent number of the whole genome, F_G , based on the formula derived by Lucas *et al.*⁷⁾

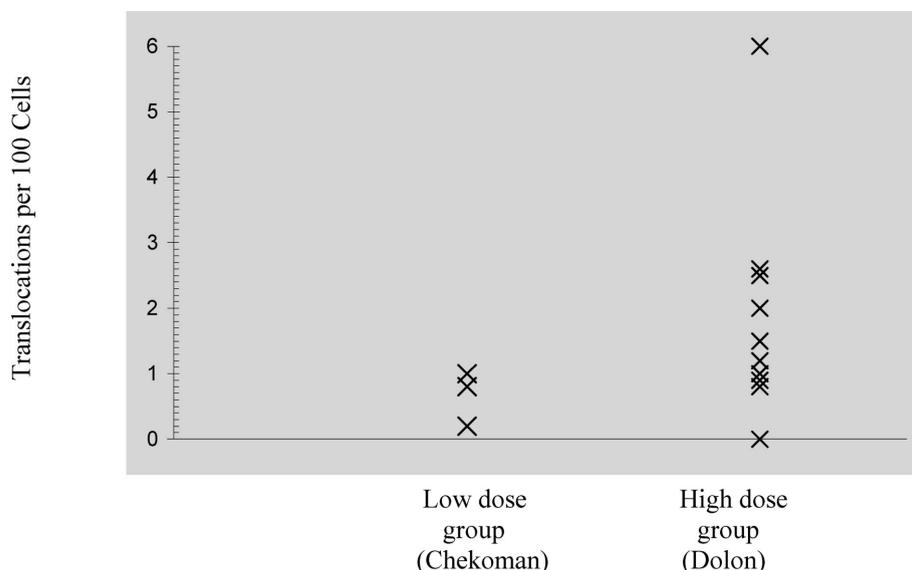


Fig. 1. The number translocations per 100 cells for two groups of women living in Dolon and Chekoman.

$$F_G = \frac{F_p}{2.05f(1-f)} \quad (1)$$

where f is the fraction of the painted chromosomes. The per-

centage of the whole-genome equivalent aberrations is presented in the last column of Table 1. The average number of aberrations in Dolon samples was found to be 1.6 ± 0.2 per 100 cells. The samples from the 5 Chekoman residents

Table 1. Chromosome aberrations in blood samples collected from the residents of Dolon and Chekoman.

Group	Subject	Chromosomes analyzed	Cells scored	Number of aberrations	Total genome equivalent number	Number of aberrations in 100 cells
Dolon (High dose)	D1	1&2	964	8	26.4	2.7
	D2	1&2	221	0	0	1
		2	1604	3	18.6	
	D3	1&2	3348	11	36.3	1.1
	D4	1&2	3567	9	29.7	0.8
	D5	1&2	1105	5	16.5	1.5
	D6	1&2	132	1	3.3	2.5
	D7	1&2	181	0	0	0
	D8	1&2	164	1	3.3	2
	D9	1&2	58	1	3.3	5.7
		2	1019	6	37.2	
Total			15086	64	237.3	1.6 ± 0.2
Chekoman (Low dose)	C1	1&2	851	1	3.3	0.4
		2	3190	2	12.4	
	C2	1&2	2011	5	16.5	0.8
	C3	1&2	283	0	0	0
	C4	1&2	934	1	3.3	1.1
		2	749	2	12.4	
C5	1&2	712	0	0	0	
Total			8730	11	47.9	0.6 ± 0.18

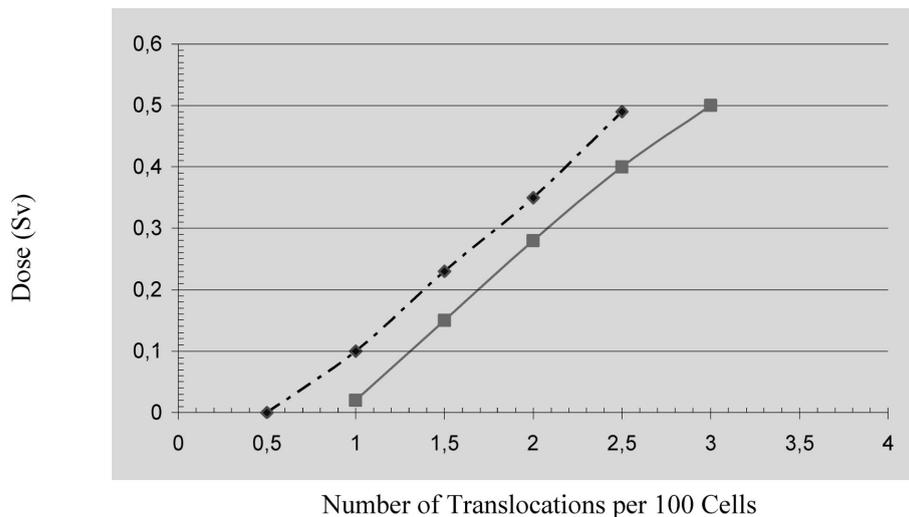


Fig. 2. Dose vs whole genome translocation frequency.

yielded an average frequency of 0.6 ± 0.18 per 100 cells. Individual as well as the average frequencies are shown in Fig. 2.

If the data from the slides without signals from chromosome #1 were excluded, the total number of cells scored for Dolon samples was 12463 and the total number of aberrations was 55. The frequency of aberrations was thus 0.44 ± 0.06 in chromosomes #1 and #2 and 1.5 ± 0.2 for the whole genome equivalent. For the Chekoman group, the total number of cells scored, excluding the slides without the signals from chromosome #1, was 4791 and the total number of aberrations was 7, yielding an aberration frequency of 0.15 ± 0.06 for the two chromosomes and 0.48 ± 0.18 for the whole genome equivalent.

The effective doses for these two groups of women living in Dolon and Chekoman were estimated based on the work of Edwards³⁾ who tabulated FISH analysis of chronic X-ray exposed human samples. The number of translocations in that table was 20% of the genome and in this paper is converted to a number corresponding to the whole genome equivalent. The relationship between the dose and the whole genome translocation frequency is shown in Fig. 2. Using the line in Fig. 2, the effective dose for the Dolon group was estimated to be 180 mSv and the aberration frequency in the Chekoman group was below the limit of detection.

DISCUSSION

Although at present cytogenetic techniques are widely used as biological dosimetry method in situations ranging from nuclear accidents⁹⁾ to space travel¹⁴⁾ the accuracy is limited by many factors particularly variation in the background frequency of stable translocations. In studies of the control group living in Livermore, California, USA¹¹⁾ the frequency of chromosome aberrations was found to increase with age and was best fitted by the formula

$$Y = 0.06154 + 0.000304 \times \text{age}^2 \quad (2)$$

As the average age of the subjects in the present study was 51 years at the time of sample collection, the background frequency was calculated to be 0.85 per 100 cells. As shown in Fig. 2, this value is close to the number of translocations at 0 Gy. Therefore, the line in Fig. 2 is appropriate to be applied to the group of women in the present studies.

The present studies showed a significant increase of chromosome aberration frequency for the Dolon group over the Chekoman group (Dolon village is located at a distance about 100 km from the Semipalatinsk nuclear test site).¹⁵⁾ If the frequency for the Chekoman group is used as a control one and the same dose response as in Fig. 2 is adopted (as shown as the dashed line in Fig. 2), the estimated effective dose was evaluated to be 260 mSv for the Dolon group. Since the women in the Chekoman group had also been exposed to radiation, the dose for the Dolon group could be

higher.

Despite the limitations, analysis of chromosome aberrations is the most sensitive of all biological monitors available for victims exposed to radiation decades ago.^{3,7)} Studies showed that the frequency of stable translocations persists over a period of several decades⁸⁾ and, thus, stable aberrations scored by the FISH technique provide the best tool to reconstruct the biological dose for prior exposures.⁷⁾

Other biological endpoints have also been investigated recently among the residents living in the area of the Semipalatinsk nuclear testing site. Studies of micronuclei in erythrocytes and lymphocytes in the blood of the residents showed a significantly higher frequency of micronucleated cells in the majority of individuals living in the contaminated regions as compared to those of the control group.⁴⁾ However, these studies were not designed for biological dosimetry. Studies of dicentric and rings in samples from the residents living in the area showed that the frequency of chromosome aberrations significantly exceeded the control level.¹⁾ However, these types of unstable aberrations are less indicative of doses from prior exposures.

CONCLUSIONS

A biodosimetry investigation was conducted for two villages in the area of the Semipalatinsk nuclear testing site: Dolon and Chekoman. The village of Chekoman is considered to be relatively less affected by radiation than Dolon. The life styles of the residents are similar in both villages. Analyses of chromosome aberrations in lymphocytes collected from the residents of the two villages were performed by the FISH technique. The results obtained showed that the average frequency of stable translocations for the Dolon group was significantly greater than that of the Chekoman group. The elevated level of stable translocations with the Dolon residents corresponds to a dose of about 180 mSv.

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