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# High Incidence of Micronuclei in Lymphocytes from Residents of the Area near the Semipalatinsk Nuclear Explosion Test Site

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The Semipalatinsk area is highly contaminated with radioactive fallout from 40 years of continuous nuclear testing. The biological effects on human health in this area have not been studied. Significant remaining radioactivities include long-lived radioisotopes of $^{238, 239, 400}$Pu, $^{137}$Cs and $^{90}$Sr. To evaluate the long-term biological effects of the radioactive fallout, the incidence of micronuclei in lymphocytes from residents of the area was observed. Blood was obtained from 10 residents (5 females and 5 males, aged 47 to 55 years old) from each of the 3 areas of Znamenka, Dolon and Semipalatinsk, which are about 50–150 km from the nuclear explosion test site. For micronucleus assay, PHA-stimulated lymphocytes were cultured for 72 h and cytochalasin B was added at 44 h for detecting binuclear lymphocytes. Five thousand binuclear lymphocytes in each resident were scored. The means of micronucleus counts in 1,000 lymphocytes in residents of Semipalatinsk, Dolon and Znamenka were 16.3, 12.6, and 7.80, respectively, which were higher than those of the normal Japanese persons (4.66). These values were equivalent to the results obtained from 0.187–0.47 Gy of chronic exposure to $\gamma$-rays at a dose rate of 0.02 cGy/min. The high incidence of micronuclei in residents of the Semipalatinsk nuclear test site area was mainly caused by internal exposure rather than external exposure received for the past 40 years.

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## INTRODUCTION

Over the 40 year period from 1949 to 1989, the former USSR conducted more than 450 nuclear tests, including ground, atmospheric and underground tests, at the proving ground near the city of Semipalatinsk in eastern Kazakhstan. These experiments varied considerably in type and size, and resulted in global and localized dispersal of radioactive materials. Significant remaining nuclear activity includes long-lived radioisotopes of plutonium ($^{238}$Pu, $^{239}$Pu, and $^{400}$Pu), americium ($^{241}$Am), cesium ($^{137}$Cs) and strontium ($^{90}$Sr). The atomic ratio of $^{240}$Pu/$^{239}$Pu in the soil at a depth of 10 cm was in the range of 0.024–0.125 at the nuclear test site and in the residential area, which was significantly lower than the value of 0.18 commonly accepted for global fallout plutonium. It is clear that the contamination was derived from weapons-grade Pu from atomic bomb materials themselves due to the Semipalatinsk nuclear tests[1,2]. Except for the nuclear weapon test sites themselves, most areas had the same levels of radiation dose rate, which was evaluated by gamma survey and in situ spectroscopy on the ground[3,4], as the national background levels in Japan (0.1 $\mu$Sv/h). The results of thermoluminescence dosimetry in bricks on building walls revealed significantly higher radiation exposure in 2 areas, e.g., 90 cGy in Dolon and 50–69 cGy in Semipalatinsk city[5,6]. These results indicated that fallout containing high radioactivities went through these villages from 1948 to 1989, and there was external exposure, which is different from that in the Chernobyl nuclear power plant accident. These results, which were obtained by both measurement of plutonium levels and thermoluminescence dosimetry, demonstrated significant internal and external radiation exposure in residents of these areas due to nuclear testing.

Aforementioned physical measurements are indirect dosimetry in terms of human effective doses. Direct biological dosimetry is necessary to evaluate biological effects in residents of the area near the Semipalatinsk nuclear test sites. However, biological effects on human health in residents of the area have not been well studied. In order to assess the effects of prolonged radiation on the people and the environment caused by the past nuclear tests at the Semipalatinsk nuclear test sites, we focused on radiation dosimetry and associated disease in the population. Higher incidences of esophagus, stomach, liver, lung, breast and thyroid cancer were observed among residents in the most heavily exposed zones of the Semipalatinsk area compared with other non-contaminated areas[7].

Micronucleus assay and chromosome aberration analysis of lymphocytes from humans exposed either *in vivo* or *in vitro* to ionizing radiations have proven to be valuable tools of dose estimation. Chromosome analysis requires a high level of skill, and micronucleus scoring has become a popular assay for detecting clastogenicity. In a given moment of time, a greater number of cells can be scored for chromosome analysis, increasing the power and thus the sensitivity of the analysis[8–10]. Cytokinesis is blocked by cytochalasin B, which leads to binucleated cells in the first division[11]. This has led to several investigations comparing the induction of chromosome aberrations and micronuclei in binucleated lymphocytes exposed to ionizing radiation[12–17]. In this way, the micronucleus assay is a suitable tool for use as a biological dosimeter after accidents involving partial-body irradiation[11,18,19].

## MATERIALS AND METHODS

*Subjects and samples*

Blood was obtained from 30 healthy adults with their informed consent on September, 1998. The subjects had been living in the areas in question since the above-ground nuclear tests were performed. Ten people, 5 females and 5 males, from each of the 3 different areas, Znamenka, Dolon and Semipalatinsk, were used in the present study. Znamenka and Dolon are located about 50 km and 100 km from the center of the Semipalatinsk nuclear explosion test site, respectively, and the city of Semipalatisk is about 150 km from the nuclear test site. The locations of the areas are shown in Fig. 1. Out of the 30 people, 17 were Kazakhstani, and 13 were Russian. Their ages ranged from 47 to 55 years old. All of them had experienced nuclear weapon tests. For example, the 47-year-old residents had experienced nuclear tests since 1951 and the 55-year-old residents were 5 years old at the time of the first nuclear test in 1948. All of the 30 residents were interviewed on their lifestyle using a questionnaire. Information on the questionnaire included experience of nuclear tests, previous radio- or

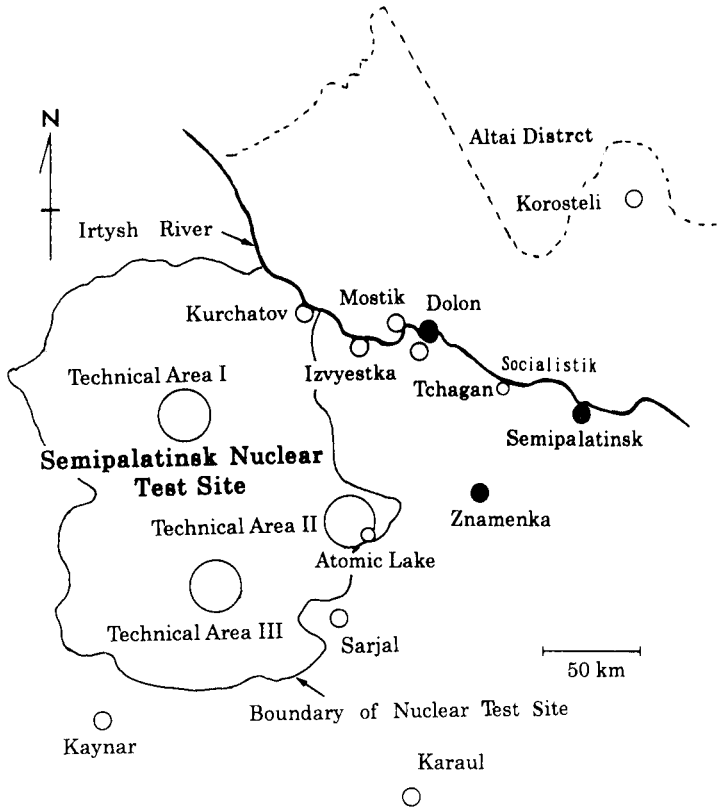


**Fig. 1.** Map of the area near the Semipalatinsk nuclear test site. Closed circles indicate the 3 sampling areas.

chemotherapy, the subject's work, smoking and dietary habits, coffee intake, food, etc. No cancer patients are included in these 30 peoples.

A 20 ml sample of whole blood drawn by a sterilized syringe from each person was immediately mixed with 20 ml of RPMI 1640 medium containing 20% fetal bovine serum (FBS; Sigma, St Louis, USA) in a 50 ml conical tube, and then 1% phytohemagglutinin (PHA; Murex Diagnostics, Dartford, UK) was added, and then it was stored in a container at 4°C. These samples were brought to Japan. Ten adult healthy Japanese persons (5 females and 5 males), who had the same age distribution as the Semipalatisk residents were used as controls because of the difficulty of obtaining sufficient quality control samples around the Semipalatinsk region, where all the areas were contaminated with residual radioactivities resulting from the nuclear tests. Eight other healthy Japanese adults, whose age ranged from 23 to 70 years old, were also used for analyzing the age distribution of micronucleus incidence.

*Culture method*

After the samples were brought to Japan, the cell suspensions were immediately washed several times with PBS, the lymphocytes separated by Ficoll gradient, and four cultures were set up using 50 ml culture flasks. One flask was cultured for 72 h for the micronucleus assay, and the 3 remaining flasks were cultured for 52 h for chromosome analysis using fresh RPMI medium plus 20% FBS.

*Micronucleus assay*

Lymphocytes were cultured for 72 h with RPMI medium containing 20% FBS plus 1% PHA for analyzing micronuclei. Samples were harvested at 72 h for the analysis. At 44 h, cytochalasin B (Sigma, St Louis, USA) was added to yield a final concentration of 5 $\mu$g/ml, and the cells were incubated for an additional 28 h. One hundred to 150 $\mu$l of cell suspension was used for each slide made with an auto-cytospin (Shandon Inc., Pittsburgh, USA). Five slides of blood from each person were prepared. The slides were stained with May-Gruenwald Giemsa solution. Micronuclei in a total of 5,000 binucleated lymphocytes were scored under the microscope with a magnification of 400X. The distribution of the number of micronuclei per cell was also observed.

*$^{137}$Cs $\gamma$-ray irradiation in vitro*

Lymphocytes were separated from 10 ml blood samples from 4 healthy Japanese persons by Ficoll sedimentation. The cells were irradiated with 0.025, 0.05, 0.1, 0.25, 0.5, and 0.75 Gy from $^{137}$Cs $\gamma$-rays at a dose rate of 0.02 cGy/min at 37°C in RPMI medium containing 20% FBS. $^{137}$Cs $\gamma$-ray doses were measured with an HU-5 ionization chamber, which is a tertiary standard dosimeter (Japanese Association of Radiological Physicists (JARP) dosimeter).

*Statistics*

Statistical analysis was performed with the Fisher exact test to compare the frequencies of cells with micronuclei. Values of $p<0.05$ were considered significant. A linear regression

line was obtained to show the relationship between incidence of micronuclei and $^{137}$Cs $\gamma$-ray exposure dose.

## RESULTS

*Incidence of micronuclei*

The incidences of micronuclei observed in 10 people from each 3 areas of Znamenka, Semipalatinsk and Dolon, and in 10 Japanese persons are shown in Fig. 2. The incidence was expressed as the number of micronuclei per 1,000 binucleated lymphocytes. The incidences (means $\pm$ 2 standard deviations) in Semipalatinsk, Dolon and Znamenka were $16.34 \pm 4.32$, $12.6 \pm 4.32$ and $7.80 \pm 3.50$, respectively, which were 1.7–3.5 times higher than that of the normal Japanese persons ($4.66 \pm 2.13$). In particular, the mean values in blood from the residents of Semipalatinsk and Dolon were significantly higher than those of the Japanese persons ($p < 0.01$) as revealed by one-tailed Fisher's exact test. The difference in the values between those of the Znamenka residents and those of the control subjects were not significantly different, but 5 in Znamenka residents showed higher values than those of any of the Japanese persons.

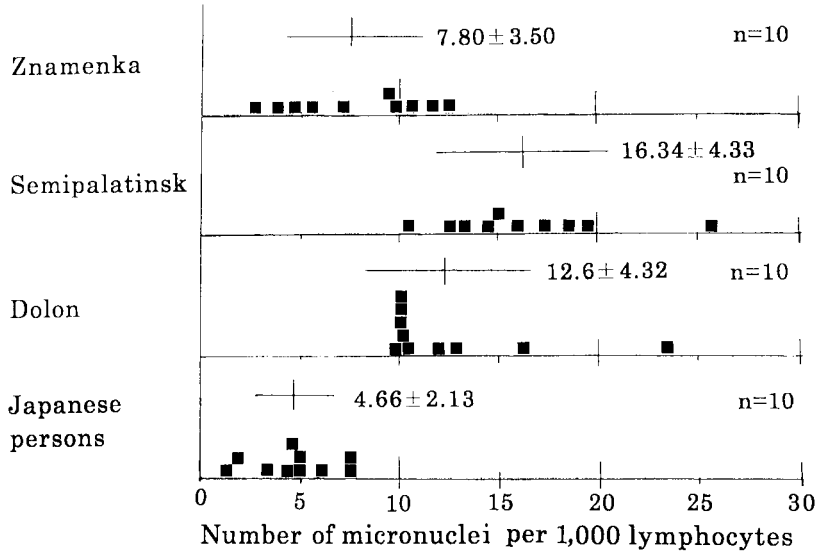


**Fig. 2.** Comparison of the incidences of micronuclei in lymphocytes from 10 residents of each 3 areas near the Semipalatinsk nuclear test site and 10 Japanese persons. Closed squares indicate the incidence of micronuclei per 1,000 lymphocytes in an individual person.

*In vitro $^{137}$Cs $\gamma$-ray irradiation and frequency of micronuclei*

The dose response relationships for incidence of micronuclei and $^{137}$Cs $\gamma$-ray exposure are shown in Fig. 3. Each point at each dose was obtained from 4–10 Japanese persons using

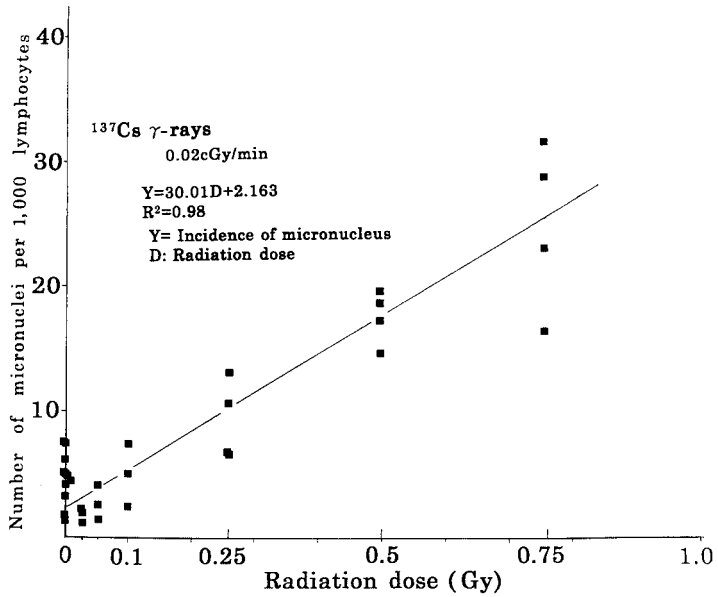


**Fig. 3.** Dose-response relationship between *in vitro* $^{137}$Cs $\gamma$-ray exposure and incidence of micronuclei in lymphocytes.

*in vitro* $^{137}$Cs $\gamma$-ray irradiation at different doses. A linear regression line was applied to show the relationship between the incidence of micronuclei and the radiation dose. The line shows $Y = 30.01D + 2.163$, where Y and D show incidence of micronuclei per 1,000 binucleated lymphocytes, and the radiation dose of $^{137}$Cs $\gamma$-rays, respectively. According to the dose response relationship, mean incidences of micronuclei in residents of Semipalatinsk, Dolon and Znamenka were equivalent to the results obtained by *in vitro* radiation exposure of $^{137}$Cs $\gamma$-rays at doses of 0.47 Gy, 0.34 Gy and 0.187 Gy exposure, respectively.

The distribution of the numbers of micronuclei per cell were also analyzed in the 4

**Table 1.** Number of micronuclei per cell in residents of 3 areas near the Semipalatinsk nuclear test sites and Japanese persons

| | Number of persons | Number of observed lymphocytes | Number of micronuclei per cell | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 | 4 |
| Residents of 3 areas near Semipalatinsk | | | | | | | |
| Znamenka | 10 | 50,000 | 49,644 | 323 ( 6.46)[a] | 32 (0.64) | 1 (0.02) | 0 |
| Semipalatinsk | 10 | 50,000 | 49,247 | 695 (13.9 ) | 54 (1.08) | 4 (0.08) | 0 |
| Dolon | 10 | 50,000 | 49,417 | 540 (10.8 ) | 41 (0.82) | 2 (0.04) | 0 |
| Japanese persons | 10 | 50,000 | 49,785 | 197 ( 3.94) | 18 (0.36) | 0 (0 ) | 0 |

[a]Number in the parentheses shows the recalculated value in 1,000 lymphocytes.

populations (Table 1). The frequency of cells with 2 micronuclei in 1,000 lymphocytes observed in the residents of Semipalatinsk, Dolon and Znamenka were 1.08, 0.82 and 0.64 respectively, which were higher than those of the Japanese persons (0.36).

## DISCUSSION

Twenty five of the 30 people living near the Semipalatinsk nuclear test site had a higher incidence of micronuclei than Japanese age-matched peoples. This higher incidence seems to be related to the exposure dose in the residents over the course of 40 years. The residents seemed to have been exposed to both internal and external radiation. Most of the internal exposure were caused by $^{238,239}$Pu and $^{240}$Pu $\gamma$-rays, $^{137}$Cs $\gamma$-rays and $^{90}$Sr $\beta$-rays, the isotopes of which have 87.7-year-, 24,100-year-, 6,570-year-, 30.2-year- and 28.8-year- half-lives, respectively, and they are incorporated into the human body. It is believed that most of the isotopes are trapped in the bones, lungs, reticuloendothelial tissue, and liver, and most were not secreted. Physical measurement of $^{238,239}$Pu, $^{240}$Pu and $^{90}$Sr in bone or other tissue from the residents of the Semipalatinsk nuclear test site is necessary. A limited number of lymphocytes flowing very near isotopes incorporated into human body were exposed to $^{238,239,240}$Pu $\alpha$-rays because the length of the track of $\alpha$-rays was extremely short, within 8 $\mu$m, which is almost the same length as the diameter of the lymphocyte nucleus.

Previously, we observed the incidence of micronuclei per 100 lymphocytes in peripheral blood from thorotrast patients and atomic bomb survivors[20]. A higher number of micronuclei was found in thorotrast patients, but not in atomic bomb survivors. Therefore, most of micronuclei in the lymphocytes seem to occur by chronic internal exposure. Stimulated mature lymphocytes have a 3 year life span[21], but a few percent of unstimulated immature lymphocytes, derived from hematopoietic stem cells, have longer life spans. The frequency of micronuclei in lymphocytes of cancer patients decreased with time 3 to 12 months after radiation-therapy[11]. This is similar to what was found for unstable type chromosome aberrations such as dicentrics and rings. These results suggest that the high incidence of micronuclei in lymphocytes found in residents near the Semipalatinsk nuclear test site was mainly caused by internal exposures rather than external exposures received over the past 40–50 years.

Furthermore, a more precise analysis in atomic bomb survivors showed that the numbers of micronuclei in erythroblasts from the bone marrow were well correlated with the exposure dose and chromosome aberration rate[20]. However, this was found only in atomic bomb survivors who were exposed to more than 1 Gy. This indicates the possibility that with a high dose of more than 1 Gy, development of micronuclei relates to radiation exposure received 40–50 years before, as well as to continuous internal exposure. Persistent cytogenetic damage produced by radiation exposure in the past leads to the formation of micronuclei in both bone marrow cells and lymphocytes. The residents of the Semipalatinsk nuclear test site area were also exposed to external radiation, especially during the 18 years between 1948 and 1966, when the first above-ground nuclear tests were performed. A recent thermoluminescence

dosimetry suggests that residents of Dolon and Semipalatinsk were exposed to accumulated external doses of 0.9 Gy, and 0.5 Gy to 0.69 Gy at most, respectively[5,6]. The sum of the internal and external exposure can also influence the high incidence of micronuclei in lymphocytes in the residents in Dolon who were externally exposed to 1 Gy. Physical measurement of the ratios of internal exposure and external exposure is important. Consequently, we may conclude that efficiency of micronucleus development in lymphocytes in residents of Semipalatinsk, who had chronic and internal exposures, is related to the total exposure dose over 40 years and the organs in which radioisotopes were deposited.

During the entire 18-year period of the above-ground nuclear testing, radioactive products mostly spread in north easterly and southern directions with the predominant winds. The Altai district in Russia is situated north easterly from Dolon and Semipalatinsk and is 300 km from the Semipalatinsk nuclear test sites. Altai residents also had high incidences of micronuclei in their peripheral blood lymphocytes and erythrocytes[22,23].

Previous exposure to dental radiation or radiotherapy and the number of cigarettes smoked and cups of coffee drunk per week may influence the rise in the incidence of micronuclei. However, in the present study no relationship between incidence of micronuclei and lifestyle, such as the number of cigarettes smoked or cups of coffee drunk per week, previous chemotherapy, was observed. Nobody received radiation therapy. Two people had received radiation exposure for dental diagnosis 2 and 3 years before the blood collection, but they did not show a higher incidence of micronuclei. An age dependent increase of micronucleus was found in the study on the 18 Japanese persons. Micronucleus is formed by loss of chromosome segment. As difference in chromosome aberration rates among races has not been observed, the base line levels of the incidence of micronuclei may be similar among races. Ideally, control samples should be obtained from Kazakhstani and Russians for future study. At this moment, we consider that internal and external radiation exposures over 40 years contributed to the higher incidence of micronucleus in residents of the Semipalatinsk nuclear test site.

The sensitivity of the micronucleus assay is about 20 cGy[24], and that of chromosome analysis is around 5 cGy[25]. Present *in vitro* experiments have indicated that the minimum detectable dose in the micronucleus assay is around 10 cGy. Chromosome analysis is a suitable tool for evaluating the biological effects of low-dose exposure. However, it has several disadvantages, e.g., requirement of the skill for scoring and time-consuming procedures. The results obtained by thermoluminescence dosimetry in Dolon and Semipalatinsk[5,6] suggest the possibility that the residents might have around 4.0–7.0% stable type chromosome aberrations, as derived from our results in atomic bomb survivors[26]. G-banding and FISH analysis also are helpful in evaluating the radiation dose from stable type chromosome aberrations[27–29]. However, the frequencies of stable type aberrations do not simply increase with accumulated dose, as was previously expected in occupationally exposed persons[30]. This chromosome analysis is not ideal for evaluating the radiation dose from protracted exposures. On the other hand, the micronucleus assay is a simple method and will be helpful in evaluating long-term chronic exposures comprised of a mixture of internal and external exposures in residents near the Semipalatinsk nuclear test sites.

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