Unstable-type Chromosome Aberrations in Lymphocytes from Individuals Living near Semipalatinsk Nuclear Test Site

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Chromosome aberration /micronucleus/Low dose exposure/Semipalatinsk.

The Semipalatinsk nuclear test site area is considered to have been highly contaminated with radioactive fallout during 40 years of continuous nuclear testing. Individuals living near the nuclear test site are considered to have been exposed to both internal and external radiation. In order to assess the effects of prolonged radiation, a chromosome analysis was performed in lymphocytes from 123 people living in three villages, Dolon, Sarjar and Kaynar, and 46 control people in Kokpekty. A micronucleus assay was also conducted in 233 people in six different contaminated villages and one control village. Frequencies of dicentric and ring chromosomes were higher in residents of the contaminated area (1.55–2.56 per 1,000 cells) than those of the non-contaminated area (0.78 per 1,000 cells). Frequencies of dicentric chromosomes with fragments were also higher in the exposed group (0.44–0.96 per 1,000 cells). Among residents of the four villages, the incidence of multiple complex chromosome aberrations (MCA) was 0.03–0.34%. Incidences of micronucleus were also higher in the exposed group (9.36–12.3 per 1,000 lymphocytes) than the non-exposed group (7.25 per 1,000 lymphocytes). The higher incidence of unstable-type aberrations such as dicentric, ring chromosomes and micronuclei found in residents of contaminated areas seems to be mainly caused by internal exposure and other factors.

INTRODUCTION

Over a period of 40 years from 1949 to 1989, the former Union of Soviet Socialist Republics (USSR) conducted more than 456 nuclear explosions, including atmospheric and underground tests, at the Semipalatinsk nuclear test site (SNTS). The total energy release of about 18 Mt equivalent of TNT is eleven hundred times that of the atomic bomb dropped on Hiroshima. The SNTS area is thus considered highly contaminated with radioactive fallout. Significant remaining activity includes long-lived radioisotopes of $^{239,240}$Pu, $^{137}$Cs and $^{90}$Sr. Radioactive clouds passed through villages during each nuclear test and they were also the source of external radiation. Therefore, individuals living near the test site are considered to have been influenced by both internal and external exposures.

Previous reports concerning the effects of radiation on residents near the SNTS based on data provided by the Defense Department of the former USSR did not have direct experimental data concerning effective equivalent dose. The Department just reported the remaining levels of radioactivity and measured some doses in settlements after nuclear explosions. These do not indicate the integrated dose for residents from all the explosions. Epidemiological studies showed a higher incidence of cancer in the esophagus, stomach, liver and lung among residents near the SNTS.

Chromosome aberrations in circulating lymphocytes are considered the most reliable indicator of radiation exposure, especially at low doses. To evaluate long-term biological effects, the establishment of biological dosimetry for the residents will be necessary. Chromosome analysis successfully applied to dosimetry for the Chernobyl nuclear accident, enabled us to evaluate accumulated external dose and internal dose. In the present study, dicentric and ring chromosomes were used in the evaluation of radiation exposure in people living in the vicinity of SNTS.

In order to assess the effects of prolonged exposure to radiation on individuals, an analysis of dicentric and ring...
chromosomes was performed in lymphocytes from 123 residents of three contaminated villages, Dolon, Sarjar and Kaynar, and 46 controls in Kokpekty. The MN assay is a simple method and the percentage of induced MN-positive cells has a high correlation with irradiated dose in the case of chronic exposures, the same as for dicentric and ring chromosomes. A micronucleus (MN) assay was also conducted in a group of 233 people mostly not the same as for the first sampling, who were in six different contaminated villages and one control village, which will be useful for confirming the results of dicentric and ring chromosomes.

MATERIALS AND METHODS

Subjects and samples

Blood was obtained from total of 380 healthy adults with their informed consent over five years from September 1998 to August 2002. The subjects had been living in the area since between 1948 and 1965 when the atmospheric nuclear tests were performed. Residents born after the underground tests were excluded from the present study. Therefore, the subjects ranged in age from 45 to 73 years. All of them had experienced atmospheric nuclear tests since 1951. 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. Most of the 123 residents for the chromosome analysis and 233 residents used for the micronucleus assay were interviewed about their experience of nuclear tests and their life style using a questionnaire. Out of the 380 patients, twenty-two were analyzed by both chromosome analysis and MN assay. No cancer patients are included in these 380 individuals. Some 123 people, 90 females and 33 male, from each of the three different contaminated areas, Dolon, Sarjar and Kaynar located 30–50 km to the east or the south of the nuclear test site, and 46 controls, 35 female and 11 male, from an uncontaminated area, Kokpekty located about 500 km to the east were examined. Kokpekty was reported to be un-contaminated. Three (female and one male) exposed residents from Znamenka and 4 (female only) from Semiplatinsk city were also examined. On the other hand, 233 people, 134 females and 99 males, from 6 different contaminated areas, Dolon, Sarjar, Kaynar, Znamenka, Socialistic, and Semiplatinsk city, and one un-contaminated area (Kokpekty) were used for the micronucleus assay. Contaminated areas, Znamenka, Socialistic and Semiplatinsk city were located about 30–150 km to the east of the nuclear test site.

Culture method

A 20-ml sample of whole blood drawn with a sterilized syringe from each subject was immediately mixed with 20 ml of RPMI 1640 medium containing 20% fetal bovine serum (FBS) in a 50-ml conical tube, 1% phytohemagglutinin (PHA) was added, and then the mixture was stored in a container at 4°C (IAEA 2001). These samples were transported to Japan. Four flasks were set up using 10-ml culture flasks. Whole blood was cultured for 52 hours using RPMI 1640 medium plus 20% FBS at 37°C with 5% CO₂ gas.

Chromosome analysis

Colcemid was added for the last two hours in the culture. The chromosome samples were cultured and fixed in 1998, 2000, 2001, and 2002. The fixed cell suspensions were stored in a freezer until slides were prepared. Slides were made by the air-dry method and stained in Giemsa solution. About 200–500 suitable metaphases from each individual were observed. Unstable-type chromosome aberrations were scored and classified under the microscope. All of the abnormal cells were photographed and notated on the photograph after microscopic observation. Multiple complex aberration (MCA) cells were excluded from the scoring of dicentric and ring chromosomes and so on. A cell was defined as having multiple aberrations if it contained more than three dicentric chromosomes plus centric rings, acentric rings, fragments, double minute chromosomes and so on.

Micronucleus(MN) assay

The cell suspensions were immediately washed several times with PBS, the lymphocytes separated with Ficoll gradient, and four flasks were set up using 10-ml culture flask and cultured for 72 hr for the micronucleus assay. Lymphocytes were cultured for 72 h with RPMI medium containing 20% FBS plus 1% PHA for analyzing micronucleus. Samples were harvested at 72 h for the analysis. At 44 h, cytochalasin B was added to yield a final concentration of 5 μg/ml, and the cells were incubated for an additional 28 h. One hundred to 150 μl of cell suspension was used for each slide made with an Auto-Cytospin. The slides were stained with May-Grünwald Giemsa solution. Micronuclei in a total of 5,000 binucleated lymphocytes per individual person where possible were scored under the microscope.

RESULTS

Incidence of unstable-type chromosome aberrations

Results of chromosome analyses of 123 peoples from the five contaminated areas of Dolon, Sarjar, Kaynar, Znamenka, Semiplatinsk city and 46 people from the un-contaminated area, Kokpekty are summarized in Table 1. Each area had almost the same age distribution. Frequencies of dicentric and ring chromosomes from lymphocytes of residents of Dolon, Sarjar, Kaynar, were 2.55, 1.69 and 1.55 per 1,000 cells, respectively, compared to 0.78 per 1,000 cells in the un-contaminated area, Kokpekty, although the differences in values between contaminated area and control area were not statistically significant. Frequencies of cells with dicentric chromosomes with fragments were also higher in contaminated areas (0.44–0.96
### Table 1. Unstable-type chromosome aberration rates in lymphocytes from residents of five villages near the SNTS and one control village.

<table>
<thead>
<tr>
<th>Villages</th>
<th>No. observed subjects</th>
<th>Age Mean ± SD</th>
<th>No. observed cells</th>
<th>No. abnormal cells (%)</th>
<th>No. Dic (per 1,000 cells) Mean ± SD</th>
<th>No. Rc (per 1,000 cells) Mean ± SD</th>
<th>No. Dic+Rc (per 1,000 cells) Mean ± SD</th>
<th>No. Dic+F (per 1,000 cells) Mean ± SD</th>
<th>No. chr. ab per ab. cell</th>
<th>No. chromatid ab. (%)</th>
<th>No. MCA cells</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contaminated areas</strong></td>
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<tr>
<td>Dolon</td>
<td>35</td>
<td>58.5 ± 5.18</td>
<td>9,794</td>
<td>(0.63)</td>
<td>62 (1.74)</td>
<td>8 (2.55 ± 4.11)</td>
<td>9 (0.92 ± 6.6)</td>
<td>1.71 (0.95)</td>
<td>70 (0.34 ± 1.1)*</td>
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<tr>
<td>Sarjar</td>
<td>48</td>
<td>56.9 ± 5.36</td>
<td>13,642</td>
<td>(1.03)</td>
<td>141 (1.17)</td>
<td>7 (1.69 ± 2.3)</td>
<td>6 (0.44 ± 3.9)</td>
<td>1.88 (1.67)</td>
<td>228 (0.1 ± 0.85)</td>
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<tr>
<td>Kaynar</td>
<td>33</td>
<td>56.9 ± 4.84</td>
<td>11,650</td>
<td>(0.34)</td>
<td>40 (1.2)</td>
<td>4 (1.55 ± 2.8)</td>
<td>8 (0.69 ± 5.0)</td>
<td>1.7 (0.74)</td>
<td>86 (0.05 ± 0.39)</td>
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<tr>
<td>Znamenka</td>
<td>3</td>
<td>49.7 ± 0.57</td>
<td>1,340</td>
<td>(3.06)</td>
<td>41 (3.7)</td>
<td>5 (6.7)</td>
<td>9 (0.6 ± 2.2)</td>
<td>1.24 (2.84)</td>
<td>38 (0.0)</td>
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<tr>
<td>Semipalatinsk</td>
<td>4</td>
<td>51.3 ± 1.62</td>
<td>803</td>
<td>(2.62)</td>
<td>21 (2.5)</td>
<td>2 (5.0)</td>
<td>4 (0)</td>
<td>1.62 (5.23)</td>
<td>42 (0.13)</td>
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<tr>
<td><strong>Total</strong></td>
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<td><strong>Control area</strong></td>
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<tr>
<td>Kokpekty</td>
<td>46</td>
<td>52.1 ± 3.34</td>
<td>14,192</td>
<td>(0.47)</td>
<td>66 (0.6)</td>
<td>2 (0.78 ± 2.2)</td>
<td>11 (0.42 ± 3.3)</td>
<td>0.8 (1.44)</td>
<td>204 (0.03 ± 0.28)</td>
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<tr>
<td><strong>All total</strong></td>
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</table>

Dic: dicentric chromosome; Rc: centric ring chromosome; F: fragment; MCA cell: cell with multiple complex aberrations; chr. ab: chromosome aberration; ab cell: abnormal cell * Significant from that of control area, P < 0.05, by χ-square test.

### Table 2. Number of micronuclei per 1,000 cells in residents of 6 areas near the SNTS and one control area.

<table>
<thead>
<tr>
<th>Villages</th>
<th>No. of persons</th>
<th>Mean obs.* cells per person</th>
<th>Mean no. of cells with MN** per person(%)</th>
<th>Mean no. of MN** per person</th>
<th>No. of MN** per 1,000 cells (± SD)***</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contaminated areas at SNT</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Dolon</td>
<td>53</td>
<td>5018.5</td>
<td>46.8(0.93)</td>
<td>50.8</td>
<td>9.36 ± 3.46</td>
</tr>
<tr>
<td>Sarjar</td>
<td>44</td>
<td>4098.3</td>
<td>38.9(0.95)</td>
<td>42.2</td>
<td>9.9 ± 3.62</td>
</tr>
<tr>
<td>Kaynar</td>
<td>43</td>
<td>5132.4</td>
<td>50.2(0.98)</td>
<td>55.8</td>
<td>9.87 ± 3.62</td>
</tr>
<tr>
<td>Semipalatinsk</td>
<td>42</td>
<td>4734.2</td>
<td>61.8(1.31)</td>
<td>61.8</td>
<td>12.3 ± 3.94</td>
</tr>
<tr>
<td>Znamenka</td>
<td>10</td>
<td>5112.1</td>
<td>55.9(0.7)</td>
<td>35.9</td>
<td>7.1 ± 3.0</td>
</tr>
<tr>
<td>Socialistic</td>
<td>20</td>
<td>4348.4</td>
<td>31.8(0.73)</td>
<td>34.1</td>
<td>7.3 ± 3.08</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
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<td></td>
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<tr>
<td><strong>Control area</strong></td>
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</tr>
<tr>
<td>Kokpekty</td>
<td>21</td>
<td>5034.5</td>
<td>36.5(0.72)</td>
<td>38.5</td>
<td>7.25 ± 2.14</td>
</tr>
<tr>
<td><strong>All total</strong></td>
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</table>

*– obs. – observed
**–MN – micronucleus
*** – Not significant from that of control area by χ-square test.
Incidence of micronuclei respectively. Adult Japanese were irradiated have used a conventional cytogenetics approach to evaluate exposure to ionizing radiation for the evaluation of the biological effects of chronic low-dose radiation. Many authors have used a conventional cytogenetics approach to evaluate the consequences of chronic low-dose contamination. For example, significant increase in the dicentric chromosome aberration rate was observed among residents of contaminated areas in the Chernobyl accident and Chernobyl cleanup workers, and among residents settled in houses with chronic low-dose radiation exposure.

Testa et al. obtained similar results to ours, in which 21 people in Dolon village had 2.6 dicentric and centric ring chromosomes per 1,000 cells, quite similar to our data from Dolon. Their values in Dolon are within the range of the dicentrics reported in large human population studies on elderly subjects: 0.85 and 0.56 per 1,000 cells. However, the aberration rates were in general slightly higher than those in Bryansk district of Russia and the typical control rate after several years from the Chernobyl accident. The rate of dicentric and centric ring chromosomes was 0.81 and 0.51 per 1000 cells in the contaminated areas and control area, respectively. The present study showed an increase of unstable type chromosome aberrations in the peripheral lymphocytes of residents of contaminated areas at the SNTS, although their values were not statistically significant.

The mean life span of T-lymphocytes is generally expected to be 3.5 years. Moreover, half-lives as short as 110-160 days have been reported in the Goiana accident victims. In our case, samples were obtained after long-term exposure from nuclear weapons testing. In the case of chronic exposure to low doses of ionizing radiation, the accumulation of unstable-type chromosome aberrations together with their decline makes it absolutely impossible to reconstruct the dose. On the basis of dicentric and ring chromosomes, the mean excess radiation dose of residents at the SNTS is likely to be less than 250 mSv, which is consistent with the radiological countermeasures carried out in the area. Yamamoto et al. showed that the ratio of $^{239}$Pu/$^{238}$Pu in the soil at a depth of 10 cm was in the range of 0.024–0.125 in residential areas, which was significantly lower than the commonly accepted value of 0.18 for the global fallout of plutonium.

When evaluating the effects of chronic low-dose radiation on health, confounding factors such as smoking and medical exposure must be taken into consideration. In the interpretation of the results of the chromosome aberration data from the contaminated area, the main difficulty is related to quality control with respect to age, habitation, smoking, drinking, medical exposure and life style and race differences and time of sampling and so on. Moreover one has to reconsider peoples life style and examine confounding factors such as smoking, the drinking of underground water, and so on, although the only source of information was local interviews.

The incidence was compared with that in a Chernobyl study, in which 2 of 37,543 cells (0.005%) scored from the control area and 6 of 40,707 cells (0.015%) scored from the contaminated area. The mean percentage found in residents

**Incidence of micronuclei**

The incidences of micronuclei in 233 people of six contaminated areas, Kaynar, Dolon, Sarjar, Semipalatinsk city, Socialistic and Znamenka, and the un-contaminated area, Kokpekty are summarized in Table 2. Mean incidences in the five contaminated areas, (9.36–12.3 per 1,000 lymphocytes) were also slightly higher than in the un-exposed group, Kokpekty (7.25 per 1,000 lymphocytes), although the differences between contaminated area and control area were not statistically significant.

Individual duration of residence is widely distributed from 4 to 73. The relationship between years of residence and percentage of chromosome aberrations was also analyzed, but no correlation was observed ($R^2 = 0.012$).

In our previous study, lymphocytes isolated from healthy adult Japanese were irradiated in vitro with $^{60}$Co γ-rays at a dose rate of 20 mGy/min, and we obtained a radiation dose response curve for the relationship between chromosome aberration rates and radiation dose. The numbers of dicentric plus centric ring chromosomes per 1,000 cells at doses of 0.25 Gy and 0.5 Gy were 25 and 99, respectively, although their values are slightly higher than that of the IAEA (2001) report. According to the dose-response relationship, the mean incidence of dicentric plus centric ring chromosomes in residents of Dolon, Sarjar and Kaynar was consistent with the results obtained following exposure in vitro of $^{60}$Co γ-rays at an equivalent dose of less than 0.25 Gy, respectively.

**DISCUSSION**

Unstable-type aberrations such as dicentric chromosomes and ring chromosomes are a sensitive and more reliable indicator for radiation exposure than translocation. Dicentric chromosomes can essentially be considered hallmarks of exposure to ionizing radiation for the evaluation of the biological effects of chronic low-dose radiation. Many authors have used a conventional cytogenetics approach to evaluate
of the four areas near the SNTS by us was 0.03–0.34%, remarkably higher than the values in Chernobyl regions, although the reason is not clear. The difference in the MCA cell frequencies between residents at Dolon and control area in Semipalatinsk were statistically significant. Sevankaev and coworkers discussed the etiology of the cells they observed aberrations in, even in non-exposed persons at low frequencies, and called them “rogue cells.” 22-24 It is unlikely that the MCA cells seen in residents of the SNTS area would be a direct result of radiation exposure.

Translocations, which persist for some time after exposure, are believed to be superior to unstable-type aberrations such as dicentric chromosomes in detecting old or chronic exposure. However, the incidence of translocation was not high in residents of Semipalatinsk or Chernobyl. 14,25 The validity of chromosome analyses using translocation for evaluating biological effects in the case of chronic low-dose external or internal irradiation is not certain. The thermoluminescence dosimetry in bricks of walls revealed significantly higher radiation exposure in two areas, 0.9 Gy in Dolon and 0.6–0.69 Gy in Semipalatinsk city. 20 To obtain more information about the equivalent dose in residents of Dolon and other areas, an analysis of stable-type chromosome aberrations is now underway using the FISH method.

MN is formed from chromosome loss or loss of a part of the chromosome. However, MN is also induced by etiological agents like Epstein-Barr virus and so on. 25 The sensitivity of dicentric and ring chromosomes is higher than that of micronucleus assay. Therefore, the micronucleus assay may not be a good indicator for evaluating low-dose radiation-induced biological effects in later observation. More samples were collected in the present study. Results of the present MN analysis are fully consistent with our previous results. 6 Cells with MN will be eliminated during the 3–5 year life span of the lymphocyte. Therefore, the higher incidence of MN in contaminated areas in Semipalatinsk may be caused by internal exposure and so on, the same as dicentric and ring chromosomes. The reason why there is a higher incidence of the unstable type of dicentric and ring chromosomes and MN in lymphocytes from residents at the contaminated area is unknown. Other possible mechanisms may be related to inductions of chromosome aberrations in long-lived lymphocytes and chromosomal instability in descendants of radiation-exposed cells.

In conclusion, the relatively low level of chromosome aberrations in the residents is consistent with the results of the environmental measurements. The higher incidence of unstable-type chromosome aberrations and micronucleus found in residents of contaminated areas seem to be caused by internal exposure and so on.

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