Frequencies of Dicentric Chromosome and Translocation in Lymphocytes from Residents in Radio-contaminated Villages near Semipalatinsk Nuclear Explosion Test Sites

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Abstract

More than 400 above-ground and underground nuclear explosion tests were conducted at Semipalatinsk nuclear explosion test site (SNETS). The significant radioactive substances was released and radioactive plumes moved on villages at the time of explosion test, then residents in villages near SNETS are considered to be exposed internally and externally. In order to assess the biological effects on residents, frequencies of chromosome aberrations in peripheral blood lymphocytes were observed in 116 residents living in 3 villages near SNETS and 46 residents in a non-contaminated village by conventional Giemsa staining method and fluorescent *in situ* hybridization (FISH). Frequencies of dicentric and ring chromosomes in residents from the 3 villages were 1.5-2.55 per 1,000 cells, comparing to 0.78 in control area. Residents in contaminated areas had more complex chromosome aberrations. Frequencies of stable-type chromosome aberrations such as translocation were detected in 0.1% and 0.07% in 2 contaminated villages, while 0.02% in control area. More sensitive FISH analysis of chromosome subset revealed higher frequency of translocation (2.17%) in a resident. Multiple chromosome aberrations (MCA) involving more than 10 abnormal chromosomes were found in 0.12-0.42 % of observed cells among all villages, irrespective of radio-contamination. These results indicate that residents living adjacent SNETS were exposed internally and externally, but their estimated external doses were less than previously estimated 140-440 mGy, though how internal exposure was contributed to the higher incidence of chromosome aberrations is unclear.

Introduction

The Semipalatinsk nuclear explosion test sites (SNETS) is a highly contaminated area with radioactive fallout that is due to more than 456 substantial nuclear explosions, including atomosphelic and underground tests over period of 40 years from 1949 to 1989 by the former Union of Soviet Socialistic Republics in Semipalatinsk, Kazakhstan Republic. Significance remaining long lived radionuclides of ^{239,240} Pu, ^{137} Cs and ^{90} Sr has been reported in SNETS areas [1]. Residents were repeatedly exposed to radioactive plumes passed over villages during nuclear explosion tests. Then, residents were considered to be continuously exposed to both external and internal exposures for a long time. Epidemiological studies showed a higher incidence of cancer in the esophagus, stomach, liver and lung among residents [2,3].

Unstable-type chromosome aberrations (Cu) such as dicentric chromosome and ring chromosome will

be sensitive and more reliable indicator for radiation exposure, even at low dose and low-dose-rate. Frequencies of dicentric chromosome in lymphocyte has been observed in chronically exposed individuals and populations such as clean-up workers of Chernobyl nuclear accident, residents in radio-contaminated apartment houses in Taiwan and nuclear facility workers and medical technologists [4-8]. In contrast, cell with stable-type of chromosome aberrations (Cs) such as translocation can persist for a longer term after irradiation, then translocation might be superior to dicentric chromosomes to estimate accumulated dose in chronic exposure as well as exposure dose at the time of past acute exposure. In present study, we analyzed frequencies of dicentric chromosome and translocation and the spectrum of the chromosome aberrations in 116 residents living three villages adjacent to the SNETS.

Materials and Methods

1) Subjects and blood culture method

Subjects were 162 healthy adults with their informed consent from 1998 to 2002. Out of them, 116 subjects (84 females and 32 males) had been living continuously in the area since between 1948 and 1965 when the atomospheric nuclear tests were performed. The residents were from three different radio-contaminated villages in the vicinity of SNETS (Dolon, Sarjar, and Kaynar,) and 46 control residents (35 female and 11 male) from non-contaminated area, Kokpekty, where locates about 700 km away east from SNETS. Three more contaminated villages, Dolon, Sarjar and Kaynar are about 100 km away east or south from the center of SNETS. All subjects ranged in age from 45 to 73 years at the time of sampling. Residents who were born in 1939-1949 in these villages were 0-10 years old at the time of the first nuclear explosion test in 1949. For example, the 55-year-old resident was 5 years old at the time of the first nuclear tests. Cases with chronic thyroiditis who showed high anti-thyrogloblin anti-body values (TgAb $\geq$ 0.4 U/ml) were excluded from present study.

The 10 or 20 ml whole blood drawn from 162 healthy subjects was immediately mixed with 20 ml RPMI 1640 medium containing 20 ml fetal bovine serum(FBC) and 1 % phytohemaggulutinin (PHA) in 50 ml sterilized conical tube and stored at 4℃. These samples were transferred to Japan. The blood transportation method is presently modified to be more suitable for chromosome analysis [9, 10]. Four 10 ml culture flasks were set up and their blood were cultured for 52h using fresh RPMI1640 medium plus 20% FBS. Colcemide was added for the last 2h before harvesting. Cells were treated with hypotonic solution and fixing solution according to standard protocol. Chromosome metaphases were prepared by air dry method and stained in Giemsa solution. Unstable-type aberrations (dicentric, ring chromosomes and fragments) were observed under microscope in about 200-500 well-spread metaphases from each subject. All of the abnormal metaphases were photographed. Translocation, inversion and deletion were analyzed on the photos.

2) *Fluorescence in situ hybridization (FISH) method of chromosome subsets*

Peripheral blood lymphocytes from a 60 year old man, who stays in Sarjar without movement since 1940 when he was born, was used for FISH analysis. Whole chromosome specific painting (WCP) probe (Vysis, Naperville, IL, USA) were used for metaphase FISH. We applied two-colors painting of chromosomal subsets using different chromosomes, as WCP probes for chromosomes 1, 2 and 4 were

shown as rhodamine derivative spectral orange and WCP probes for chromosomes 6, 7 and 9 were FITC derivative green. These two kinds of probes were mixed and hybridized. Our FISH protocol has been published previously [11]. Painted chromosome aberrations with these WCP probes were classified according to the protocol for aberration identification [12]. Furthermore, we calculated the expected frequencies of translocation in 23 pairs of chromosome from observed translocation frequencies using Lucas's formula [13]: Frg = 2.05[fr(1-fr)+fg(1-fr)-frfg]Fg, where Frg and Fg are observed and calculated frequency translocation, respectively, and fr and fg are the percentages of the genome stained in red and green, 0.224 and 0.157 in 6 pairs of chromosomes,1, 2, 4, 6, 7 and 9 contains 38.2% of the whole genome [14].

Results

1. Frequencies of dicentric chromosome aberration

Results on chromosome aberrations in 116 residents from 3 villages (Dolon, Sarjar and Kaynar) near SNETS and 46 residents from control village, Kokpekty, are summarized in Table 1. Each village had similar age distribution(58.5±5.18 in Dolon; 56.9±5.36 in Sarjar; 56.9±4.84; 52.1±3.34 in Kokpekty) . Numbers of dicentric chromosome (Dic) in lymphocytes of residents of Dolon, Sarjar and Kaynar were 1.74, 1.17 and 1.2 per 1,000 cells, respectively, which were 2-3 times higher than 0.6 in control residents. Frequencies of dicentric and ring chromosomes (Dic+Rc) in 3 villages were 1.55-2.55 per 1,000 cells, comparing to 0.78 in control area. Also, the numbers of cell with both dicentric chromosome and fragment (Dic+ F) in a metaphase of residents of Dolon, Sarjar and Kaynar were 0.92, 0.44 and 0.69 per 1,000 cells, compared to control area (0.42). Residents in most contaminated area, Dolon, had about two times higher aberration(Dic+F) than control area. Numbers of chromosome per abnormal cell were 1.71, 1.88 and 1.7, compared with 0.8 of the control residents, which indicating that more complex chromosome aberrations were found in the residents of the three contaminated villages. Interestingly, metaphases with multiple complex chromosome aberrations(MCA), which contain more than 10 abnormal chromosomes including tricenric, dicentric, ring chromosomes, fragments and double minute chromosome in a metaphase, were observed in 0.34 % of analyzed metaphases in a contaminated village, Dolon, compared to 0.1, 0.05 in two contaminated area, Sarjar and Kaynar, and 0.03 control area, Kokpekty (Table 1). Representative metaphases with MCA are shown in Fig.1. Metaphases with MCA were excluded from scoring dicentric and ring chromosomes, because it is considered not to be radiation-induced.

2. Frequencies of trasnlocation

As shown in Table 2(a), Cs cells also observed higher incidences in residents living in the contaminated areas. Out of total 162 residents, 63 residents (28 from Dolon, 17 from Sarjar, and 18 from control area, Kokpekty) who were analyzed in 2001-2002, were used for present translocation analysis using conventional Giemsa staining method. Cs cells with either or both translocation, inversion or deletion (t + inv + del) were identified on the photos. The frequencies of residents from Dolon and Sarjar were 0.1% and 0.07%, respectively, which were higher than 0.02% in control area, Kokpekty. Frequencies of each chromosome aberration of translocation, inversion and deletion were 0.6%, 0.2 % and 0.4 % in Dolon, and were 0.4%, 0.2% and 0.4 %in Sarjar, whereas 0, 0 and 0.2 % in control area, Kokpekty. FISH

analysis of chromosome subset with two colors is more sensitive than conventional Giemsa staining method, then we applied the method to a subject of the 17 residents in Sarjar to detect precise frequency of translocation in lymphocytes. Ten metaphases (0.99%) were detected to have translocation in 1,008 observed metaphases [Table 2(b)]. Expected frequency of translocation in the man was obtained as 2.17 % by calculation with the Lucas's formula, which was higher than those obtained by conventional Giemsa staining method.

Table 1. Frequencies of chromosome aberrations in lymphocytes from residents in three contaminated villages, detected by conventional Giemsa staining method

Villages	subjects	Obs. cells	Ab. cells (%)	Dic (per 1,000 cels)	Rc	Dic+Rc (per 1,000 cells)	Dic+F (per 1,000 cells)	Comlexity (No. chr. ab. per ab. cell)	MCA cells (%)
Dolon	35	9,794	62(0.63)	17 (1.74)	8	25(2.55)	9(0.92)	1.71	33(0.34)
Sarjar	48	13,642	141(1.03)	16 (1.17)	7	23(1.69)	6(0.44)	1.88	13(0.1)
Kaynar	33	11,650	40 (0.34)	14 (1.2)	4	18(1.55)	8(0.69)	1.7	6(0.05)
Kokpekty	46	14,192	66 (0.47)	9 (0.6)	2	11(0.78)	6(0.42)	0.8	4(0.03)

Obs: observed, Ab: abnormal, Chr.: chromosome, Dic: dicentric chromosomes, Rc: centric ring, F: fragment

Table 2 (a) Frequencies of translocation in lymphocytes from residents in two contaminated villages, detected by conventional Giemsa staining method

Villages	Subjects	Obs. cells	Ab. cells(%)	Total (t+inv+del) (%)	Trans (%)	Inv	Del
Dolon	28	9,000	78 (0.87)	11 (1.2)	5(0.6)	2(0.2)	4(0.4)
Sarjar	17	5,150	20 (0.39)	5 (1.0)	2(0.4)	1(0.2)	2(0.4)
Kokpekty	18	6,600	7 (0.11)	1 (0.2)	0	0	1(0.2)

Obs: observed, Ab: abnormal, Trans: translocation, Inv: inversion, Del: deletion

(b). Frequency of translocation in lymphocytes from a resident in contaminated area, Sarjar, detected by FISH

Village	Subject	Obs. cells	Ab. cells (%)	Obs. trans	Calculated trans (%)*	Rc	Ace. ring
Sarjar	1	1,008	12 (1.19)	10 (0.99)	(2.17)	2	2

*Number of translocations in whole genomes equivalent was calculated using Lucas's formula. Obs: observed, Ab: abnormal, trans: translocation, Rc: centric ring, Ace ring: acentric ring

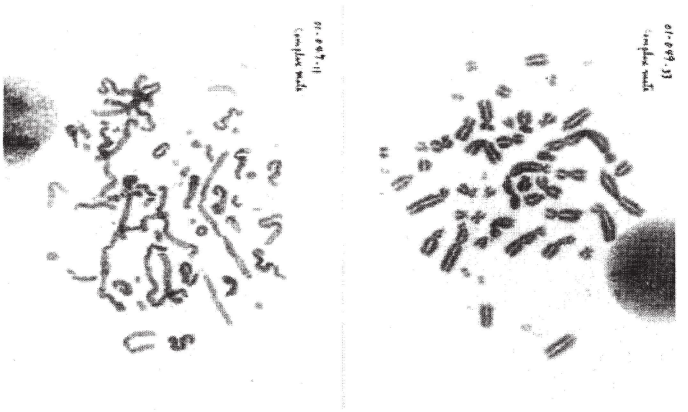

Figure 1 Representative two metaphases with MCA from independent subjects. Multiple abnormal chromosomes such as tricentric, dicentric, ring chromosomes and fragments are shown in a metaphase.

Discussion

Testa et al. obtained quite similar result on frequencies of dicentric and ring chromosome (Dic+Rc) to ours (2.55 in 1,000 cells), in which 21 subjects in Dolon village had 2.6 in 1,000 cells [15]. Another two reports on the observation in 80-100 subjects showed that mean numbers of dicentric and ring chromosome were 2.3 and 4.4 in 1,000 cells [16, 17]. These Dic +Rc values of 2.3 and 2.6 in residents near SNETS were approximately 2.9 and 4.4 times higher than background values (0.85 and 0.56 per 1,000 cells) observed in large number of non-exposed UK and Japanese persons, respectively [6,18]. A metaphase with both dicentiric chromosome and fragment (shown as Dic+F in Table 1) is considered not to have entered any cell division after exposure. The lymphocyte with such chromosome aberration is presumed to come from memory cells preserved long-term in stem cells, or to be just after exposure. The incidence of Dic+F in residents of most contaminated area, Dolon was approximately two times higher than that of control area, These cytogenetic results are indicating that residents living near SNETS have been more exposed externally or internally. Another possibility will be taken into consideration for the reason why higher incidences of unstable-type (Cu) aberrations such as dicentric and ring chromosomes (Dic+Rc), dicentric chromosomes (Dic) and dicentric chromosomes with fragment (Dic+F) was observed in residents of contaminated areas near SNETS. Cu cells harboring dicentric chromosome might be eliminated slower in chronic irradiation at low-dose-rate than previously observed in acute irradiation at high-dose-rate. Our recent experiment revealed that mice exposed to gamma-rays for 400 days at the low-dose-rate [20 mGy/22h/day(0.45 mGy/h)] had slower elimination of dicentric chromosome than expected, which was also much different from that of acute irradiation (unpublished result). Also, the

reason why chronically exposed persons at low-dose-rate such as nuclear facility workers, radio-medical technologists, clean-up workers of Chernobyl accident and so on have always higher incidences of dicentric chromosome than non-exposed persons [6,18] could be explained by same reason. Higher incidence of micronuclei, which mostly developed by radiation-induced chromosome break, found in residents in SNETS would be caused from same reason [19].

Lymphocytes with MCA are called as "rogue cells"[20]. The percentages of MCA in residents of SNETS were 0.05-0.34%, which were higher than the values of Chernobyl regions [21]. It is unlikely that MCA found in residents of SNETS region caused from direct radiation-induced damage. MCA has been etiologically discussed that it would be occurred by endemic virus because it is found worldwide even in non-exposed persons at a small incidence [22].

Chromosome aberrations is sensitive indicator for biodosimetry, specifically at low dose or low-dose-rate range, but they occur spontaneously and frequencies of translocation dramatically increase with age [23, 24]. Thus, translocation will be less sensitive indicator for biodosimetry for chronic exposure than dicentric chromosome. In present study, frequencies of translocations and Cs cells (t+ inv+del) in a resident near SNETS was 2.17 % by FISH and 0.6-0.8% by conventional Giemsa method and these values were higher than residents of non-contaminated area. Frequencies of translocations in residents near SNETS have been observed by three authors using FISH method of chromosome subset with single color [25-27], but their obtained results were discrepant. A report showed a significant result that frequency of translocation in 10 residents of Dolon was 1.6 %, comparing to 0.6% of control area [25], but other two reports had no significant results between contaminated and non-contaminated areas [26, 27]. These values detected by FISH method are within the range of elder control persons [24]. Confounding factors such as smoking, medical exposure, habitation, drinking and life style must be influenced with the frequencies of translocation in the evaluation of health effects of low-dose or low-dose-rate radiation.

Dicentric chromosomes increased in linear with dose increment in mice long-term irradiated at low-dose-rates [20 mGy/22h/day(0.91 mGy/h) and 1 mGy/22h/day(0.045 mGy/h)][28], which suggesting that dicentric chromosome will be a useful biodosimetor in chronic exposed peoples. Chromosome analysis in residents of high background radiation area in China showed a similar result [29].Then, frequencies of chromosome aberrations detected in residents of Dolon (2.55×10^{-3} for dicentric and ring chromosomes; 1.74×10^{-3} for dicentric chromosomes) in present study were used for calculation of external dose using chromosome aberration rate and dose response curves of *in vitro* acute or chronic ^{137}Cs gamma ray irradiation with lymphocytes [30], where the value of 8.5×10^{-4} was used for constant (*c*) as background value [18]. We estimated external doses in residents of Dolon as 37.4 and 33.8 mGy of gamma-ray equivalent, which were less than previously estimated as around 140-440 mGy at Dolon by physical methods such as luminescence dosimetry in brick, calculations using soil contamination, frequencies of translocation and electron spin resonance (ESR) [25, 31-35]. However, our present results suggest that estimated external doses of the residents were less than the physically estimated dose range. Our present results did not support the previously estimated external exposure dose, although it is remained to be resolved that the validity of chromosome analyses using dicentric chromosome or translocation for evaluating biological dose in chronic low-dose external or internal exposure is uncertain.

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