Cytogenetic biodosimetry for Fukushima travelers after the nuclear power plant accident: no evidence of enhanced yield of dicentrics

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Individuals who traveled to contaminated areas after the Fukushima nuclear accident have concerns about the health effects. However, medical follow-up for any adverse health effects will be difficult without personal dose measurements. Cytogenetic biodosimetry is a reasonable method of assessing absorbed doses retrospectively. We analyzed dicentric chromosomes for 265 Fukushima travelers, mostly journalists and rescue workers, who had been dispatched to northeastern Japan during the nuclear emergency. As a control group, 37 healthy volunteers who had not visited Japan since the accident were enrolled. Yields of dicentrics and absorbed doses calculated from a dose-response calibration curve for travelers and the control group were compared. The cut-off level for dicentric chromosomes in the controls was 3.5 per 1000 cells. Of the 265 travelers, 31 had elevated numbers of dicentrics (High-Dics group) while 234 were below the cut-off (Normal-Dics group). All but one of the individuals in the High-Dics group also reported a significantly higher number of medical exposures to radiation within the past three years compared with the Normal-Dics or control groups. The 225 travelers with no history of medical exposure showed no difference of dicentrics yield compared to the control group. Our data indicate that Fukushima travel alone did not enhance the yield of dicentrics.

Keywords: biodosimetry; dicentrics; Fukushima travel; medical exposure

INTRODUCTION

A massive earthquake and a subsequent tsunami struck the northeastern portion of Japan on March 11, 2011 and caused extensive damage to the nuclear power plant in Fukushima. After a series of hydrogen explosions in the nuclear power plant, a significant amount of radioactive materials was released into the environment. The Korean government sent rescue workers to assist in the disaster zones [1]. The rescue workers traveled with a radiation specialist who joined them to protect them from the radiation hazard, and some of the team members were provided with personal dosimeters. The specialist monitored radiation levels and dose rates in the air intermittently. Hundreds of journalists also visited the area to report the disaster. Unlike the rescue workers, the journalists were not provided with anti-radiation gear or any professional support. Few sources of information about the level of radioactivity in the atmosphere and the range of contamination of the surrounding ecology were available.
immediately after the accident. Lack of information and several ominous reports about the possible health risks caused these individuals great anxiety about adverse health effects [2, 3]. To find satisfactory answers regarding acute or long-term health effects, we needed to estimate personal doses for these individuals. When information from personal dosimeters was available, cytogenetic analysis provided additional information about the dose, but for individuals who were not wearing anti-radiation gear during the aftermath, biological dosimetry provided the only alternative to assess absorbed doses [4].

The main issues that were raised after the accident were whether the individuals who traveled to Japan had really been exposed to ionizing radiation from the contaminated environment and whether they would have increased risks of cancer due to the radiation. The ‘linear-no threshold’ model of radioprotection assumes that no safe dose exists and that the risk increases linearly with increasing doses of radiation exposure. Although there is no significant evidence of additional risk of cancer in low-dose ranges, potential harm has been perceived by the public [5, 6].

Biodosimetry can estimate individual dose based on the level of biomarkers induced by ionizing radiation. It is an alternative in cases of suspected exposure when physical dosimeters are not available, and it offers an additional advantage in that it can reflect biological damage with regard to inter-individual variations of susceptibility. Thus, it is a step closer to predicting problems which an exposed individual may be confronted [7]. Among several biomarkers used for biodosimetry, dicentric chromosomes are considered the most sensitive and specific indicators of dose in recently overexposed individuals [8]. The aim of this study was to investigate whether Fukushima travel enhanced the yield of dicentric chromosomes, and to provide proper guidance for management.

MATERIALS AND METHODS

Participants and controls
This study enrolled 265 individuals who visited our radiation emergency clinic between March 21 and June 15, 2011. Most of the individuals enrolled were journalists or rescue workers who had been dispatched to northeastern Japan after the accident at the Fukushima nuclear power plant. Each participant was tested for dicentric chromosomes in peripheral lymphocytes and was asked to complete a questionnaire that included information about activities and duration of stay in Japan, distance from the nuclear power plant during the visit, and past medical history, including diagnostic and therapeutic radiology. The control group comprised 37 healthy volunteers who had not been in Japan since the nuclear power plant accident. All of the participants provided informed consent for their enrollment and for donating leftover specimens for future research. The Institutional Review

Analyzing dicentric chromosomes
Whole blood samples from the participants were cultured immediately after collection. The process of culturing and staining cells was performed according to the technical specifications recommended by the IAEA [8]. Briefly, 1 ml of heparinized whole blood was cultured for 48 h in 9 ml of RPMI-1640 medium supplemented with 20% (v/v) fetal calf serum, 2% phytohemagglutinin and antibiotics. Colcemid at a concentration of 0.07 µg/ml was added 24 h before harvesting. After hypotonic treatment with 0.075M KCl, the cells were fixed in a 3:1 solution of methanol-acetic acid. The cells were prepared on a slide and stained with Giemsa, and the number of dicentric chromosomes and acentric fragments was scored per 1000 metaphase cells. The frequencies of different structural aberrations of the chromosome-type and of the chromatid-type were also recorded, but not used for dose calculation.

Dose estimation
The absorbed dose for each of the individuals tested was calculated from the measured yield of dicentrics by fitting to a dose-response calibration curve that we had previously constructed (Fig. 1). In brief, for the calibration curve, $^{60}$Co was used as a source at a dose rate of 0.5 Gy/min. A linear-quadratic curve containing 10 dose points (0, 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4 and 5 Gy) was constructed with 95% confidence intervals based on the data of yield and distribution of dicentrics for each radiation dose. The limit of detection of the estimation was 0.1 Gy and the equation is:

$$Y = C + aD + bD^2$$  \(1\)

Fig. 1. The dose-response calibration curve with 10 radiation doses and corresponding upper and lower 95% confidence limits.
Coefficient $C = 0.00146$, $\alpha = 0.02688$ and $\beta = 0.07171$.

Correlation matrix $kC = -0.22242$, $kC\beta = 0.11617$ and $k\alpha\beta = -0.72451$.

Statistics
To assess the statistical differences between the Fukushima travelers and the negative controls, and between the High-Dics or Normal-Dics groups of Fukushima travelers, we conducted an independent sample $t$-test. $P$-values $< 0.05$ indicated statistical significance in all variables. Data analysis was performed with the Statistical Package for the Social Sciences (SPSS) version 12.0 (SPSS Inc., IL, USA).

RESULTS
Control group and the cut-off level for background yield of dicentrics
The control group consisted of 24 men and 13 women aged 27 to 49 (mean, 34.2) years. No individuals in the group had traveled to Japan since the nuclear power plant accident, but 4 individuals had medical histories of CT scans of chest or abdomen (0.1 average exposure per person) in the past three years. The mean number of dicentric chromosomes per 1000 cells was 1.3 and the standard deviation (SD) was 1.1 (Table 1). The cut-off level for background yield of dicentrics was set at 3.5 per 1000 cells, as mean + 2SD, to distinguish individuals with enhanced yield of dicentrics.

High-Dics group vs Normal-Dics group
The Fukushima travelers were stratified into two groups by the established cut-off level for background yield of dicentrics. Out of 265 travelers, 31 had enhanced yields of dicentrics versus the cut-off (High-Dics group), while 234 were equal to or below the cut-off (Normal-Dics group). The mean duration of stay in Japan and the closest distance from the nuclear power plant to the areas where the individuals stayed were not significantly different for the High-Dics and Normal-Dics groups ($P = 0.18$ and 0.59, respectively). No travelers entered the evacuation radius of 20 km or experienced acute radiation syndrome. The mean age and the average number of CT scans per person were significantly higher in the High-Dics group than in the Normal-Dics group ($P < 0.05$) (Table 1).

Characterization of the High-Dics group
The number of dicentrics observed in the High-Dics group ranged from 4 to 18 per 1000 cells. Table 2 shows a detailed characterization of the 31 individuals in the group according to the number of dicentrics observed. Participants 1 and 2, who had the highest levels of dicentrics, had recently received radiotherapy or radionuclide therapy for cancer treatment. Participants 3 and 4 had histories of multiple CT scans for diagnostic purposes. Participants 6 to 31, who showed slightly enhanced dicentrics, also had histories of more frequent CT scans than the participants in the Normal-Dics or control groups (Table 2).

Table 1. Comparison of characteristics between groups of Fukushima travelers stratified by yield of dicentrics

| Control group | High-Dics (>3.5/1000cells) | Normal-Dics (≤3.5/1000cells) | $P$ value$^b$ |
|---------------|----------------------------|----------------------------|--------------|
| No. of subjects (M:F) | 37 (24:13) | 31 (30:1) | 234 (208:26) | 0.007 |
| Age, mean (years) | 34.2 | 40.6 | 36.8 | 0.588 |
| Closest distance from the NPP, mean (km) | Irrelevant | 90.5 | 99.5 | 0.179 |
| Duration of stay, mean (days) | Irrelevant | 7.7 | 11.4 | 0.007 |
| No. of exposures per person$^e$, mean | X-ray | 3.0 | 3.0 | 2.7 | 0.127 |
| | CT$^d$ | 0.1 | 1.0 | 0.1 | 0.172 |
| | PET$^a$ | 0 | 0.2 | 0 | 0.000 |
| | RT or RI | No | Yes | No | 0.000 |
| No. of dicentrics per 1000 cells$^f$, mean (range) | 1.3 (0–4) | 5.7 (4–18) | 1.1 (0–3) | |
| Estimated dose, mean (mGy) | below LOD | 116.9 | 0 | |

CT = computed tomography, $F =$ female, $M =$ male, NPP = nuclear power plant, PET = positron emission tomography, RI = radioisotope therapy, RT = radiotherapy, LOD = limit of detection. $^a$As mean + 2SD of dicentrics yield in a negative control group, 3.5 was the cutoff level to divide the Fukushima-travelers into High-Dics and Normal-Dics groups. $^b$High-Dics versus Normal-Dics groups. $^c$Number of medical radiation exposures per person in the past three years. $^d$Only chest or abdominal CT scans were included. Histories of CT scans of brain or extremities declared by a few participants were excluded. $^e$PET-CT was counted as PET, not as CT. $^f$1000 cells were analyzed for every participant. Data are presented as mean number of dicentric chromosomes observed per 1000 cells.
Fukushima travelers group with no medical exposure

Since 30 out of the 31 individuals in the High-Dics group also specified histories of medical radiation exposure, the Fukushima travelers with no medical exposure history were reanalyzed as compared with a control group without any medical exposures. As shown in Table 3, yields of dicentrics and absorbed doses were not significantly different between Fukushima travelers and non-travelers, when we excluded the individuals who specified histories of medical exposure or those who did not clarify their medical exposures.

**DISCUSSION**

Since the accident on March 11, 2011 at the Fukushima Daiichi Nuclear Power Plant in Japan, several researchers have published their professional perspectives on technical, environmental and social impacts of the accident, and the medical response to the nuclear disaster, including future follow-up plans [9–13]. Our perspective is that the primary focus in any radiation disaster should be on the health effects in people who were working in, living in, or visiting contaminated areas. Most short-term morbidity and mortality caused by high doses of radiation is associated with hematologic, gastrointestinal or cutaneous complications, as were seen after the Chernobyl accident [14, 15]. In the Fukushima accident, however, to date no acute radiation sickness has been confirmed in either the general population or the nuclear power plant workers [16]. Exposure to radioactive fallout has not been proven by acute deterministic effects, but this has not eased the public’s anxiety about the potentially carcinogenic chronic effects.

The success of any future medical follow-up or epidemiological study depends on the collection of accurate information on personal dose received [13]. Biological dosimetry is

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**Table 2.** Detailed characteristics of the 31 individuals in the High-Dics group of Fukushima travelers

| Case No. | 1 | 2 | 3 | 4 | 5 | 6–31 |
|----------|---|---|---|---|---|------|
| Sex      | Male| Female| Male| Male| Male| 26 Males |
| Age (years) | 34 | 43 | 29 | 41 | 36 | 41.4 ± 4.6 |
| Cells analyzed | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| Dicentrics | 18 | 17 | 9 | 7 | 7 | 4.6 ± 1.4 |
| Estimated dose (mGy) | 328 | 314 | 187 | 148 | 148 | 96.1 ± 103 |
| Closest distance from the NPP (km) | 23 | 60 | 40 | 30 | 50 | 103 |
| No. of exposures per person | X-ray | 3 | 3 | 1 | 4 | 3 |
| CT | 3 | 2 | 5 | 5 | 0 | 0.7 ± 0.1 |
| PET | 3 | 0 | 0 | 0 | 0 | None |
| RT or RI | RI | RT, RI | 0 | 0 | 0 | None |
| Remark | Thyroid ca. | Thyroid ca. & Breast ca. | Pancreas mass | Kidney mass |

ca. = cancer, CT = computed tomography, NPP = nuclear power plant, PET = positron emission tomography, RI = radioisotope therapy, RT = radiotherapy. All numbers indicated were the means of 26 cases numbered from 6 to 31. Number of dicentric chromosomes observed per 1000 cells. Number of medical radiation exposure per person in the past three years. Only chest or abdominal CT scans were included. Histories of CT scans of brain or extremities disclosed by a few participants were excluded. PET-CT was counted as PET, not as CT.

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**Table 3.** Comparison of characteristics between the Fukushima travelers and negative controls who specified no history of recent medical exposure by CT or PET scans

| | Control group with no medical exposure | Fukushima travelers with no medical exposure | P value |
|---|---|---|---|
| No. of subjects | 33 | 225 | |
| Age (years) | 34 | 37 | 0.047 |
| No. of dicentrics (/1000 cells) | 1.2 | 1.3 | 0.690 |
| Estimated dose (mGy) below LOD | below LOD | Irrelevant |
| No. of exposures per person | X-ray CT | 3.1 | 2.2 | 0.000 |
| CT | 0 | 0 | Irrelevant |
| PET | 0 | 0 | Irrelevant |

CT = computed tomography, PET = positron emission tomography, LOD = limit of detection. Number of medical radiation exposures per person in the past three years. Only chest or abdominal CT scans were included. Histories of CT scans of brain or extremities declared by a few participants were excluded. PET-CT was counted as PET, not as CT.
a reasonable tool to assess absorbed doses, especially in circumstances where personal dosimeters were not available at the time of exposure. A dicentrics assay is currently the most sensitive and specific biodosimetry assay [17, 18]. However, average background levels reported for these assays vary from 0.5 to 10 dicentrics per 1000 cells, depending on the detailed method used in each laboratory and on local natural background radiation [17]. This disparity of normal background levels is the reason we estimated our own negative control group. The average yield of dicentrics reflecting background radiation in our negative controls was 1.3 dicentrics per 1000 cells, which was comparable with other studies [8, 17]. To obtain a reasonable specificity, we set the cut-off for background at a level of twice the standard deviation from the average (mean + 2SD).

We observed that 31 individuals had higher yields of dicentrics than the cut-off level. In the two individuals with the highest number of dicentrics (Cases 1 and 2), we determined that recent radiation or radionuclide therapy received for cancer treatments caused the additional chromosomal aberrations. While Individuals 3–31 did not report histories of therapeutic radiation exposure, their frequency of recent exposures to diagnostic radiation, particularly CT scans, was significantly higher than among the individuals in the Normal-Dics and control groups. In Individuals 3 and 4, the average frequency of exposure to radiation from CT scans was 50 times higher than the average frequency in the Normal-Dics or control groups. The results obtained from Participant 5 were difficult to interpret. In this case, the dicentrics-per-cell distribution of seven dicentrics, two of which were observed in the same cell, were clearly over-dispersed from the Poisson distribution (dispersion coefficient $\mu = 6.76$). An overdispersed distribution is indicative of a partial body exposure to a low-linear energy transfer (LET) radiation or of an exposure to a high-LET radiation. Using the Dolphin’s method [8], the theoretically estimated partial body dose in this case was 1.96 Gy. However, since this individual had stayed for eight days in areas with the closest distance to the nuclear power plant of about 50 km and had no history of medical exposures during the past three years, the observation of the cell with two dicentrics was finally considered a spurious observation.

The use of CT scans for diagnostic evaluation has been dramatically increasing over recent decades [19]. Although the widespread use of CT scans probably represents an advance in diagnostic radiology, increased exposure to radiation and the consequent cancer risk may be a public health issue [20, 21]. Our data support previous studies that have indicated an increase in levels of chromosomal aberrations after CT scans [22, 23].

The results of the present study should be interpreted in the context of several limitations. First, we did not use precisely measured doses of radiation for each CT scan that our test subjects had taken. Since this study was not originally designed to clarify the effects of CT scans, the cumulative dose from CT scans per person was not surveyed. Instead, we relied on information regarding the number of CT scans each individual had taken in the past three years and which anatomical areas had been examined. Second, chromosomal translocations should have been studied by fluorescence in situ hybridization in addition to dicentrics analysis to investigate the cumulative cytogenetic effects of past exposures from CT scans thoroughly. Unstable aberrations like dicentrics decline rapidly during the first few months after exposure, especially after high-dose irradiation [18]. The exact rate of decline, however, depends on an individual’s immunological response, which influences the turnover rate of lymphocytes, and varies extensively between individuals. Thus, the data from dicentrics analysis in our study might underestimate the effect of past CT scans. Lastly and most importantly, we could not compare our data from biological dosimetry with any individual doses from physical dosimetry. As pointed out earlier, most of our study subjects were not wearing personal dosimeters and had travelled different paths during their work. Even if directly measured doses in the atmosphere around the areas were available, they could not represent the personal doses correctly, because of the heterogeneous distribution of radioactive materials even in the same areas. Therefore, biological dosimetry was the optimal tool available to estimate personal doses and differentiate exposed individuals from non-exposed.

In spite of the limitations, our findings have indicated that there is presently no evidence of exposure among Koreans who traveled to Japan after the Fukushima nuclear accident. This is the first report of data from biological dosimetry in a general population outside of Japan that had a relatively high risk of exposure due to visiting contaminated areas. Besides our primary findings, we found that extensive use of CT scans leads to cytogenetic aberrations, which have been linked to the induction of cancer [20, 21]. Unlike the exposure of industrial workers, which can be regulated, the exposure from medical imaging is difficult to regulate. Further studies focusing on the effect of CT scans on chromosomal aberrations should follow.

We conclude that travel to Fukushima did not enhance the yield of dicentrics in Korean individuals after the nuclear power plant accident. Personal doses estimated by cytogenetic biodosimetry indicated a negligible risk of additional adverse health effects.

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