「Analysis of the CT scan-induced human chromosome aberration」
(CT スキャンにより誘発されるヒト染色体異常の解析)

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CONTENTS

LIST OF ABBREVATIONS

CT: computed tomography

HBRA: high background radiation area

Dic: dicentric chromosome

DCA: dicentric chromosome assay

PB: peripheral blood

IAEA: international atomic energy agency

FISH: fluorescence *in situ* hybridization

DNA: deoxyribonucleic acid

RT: room temperature

SSC: saline-sodium citrate

DSB: double-strand break

INTRODUCTION

Computed tomography (CT) is a very useful medical diagnostic procedure and is popularly implemented in Japan. Recent studies have reported that cancer risks associated with ionizing radiation exposure from CT scans are a concern, especially for children and young adults, because of an increase in the reported cases of leukemia, brain tumors, and other cancers following exposure[1,2]. Low-dose radiation exposure (even at doses less than 100 mSv) associated with CT scanning may induce chromosomal aberrations that may cause cancer and hematological malignancies including chromosomal translocations, deletions, and inversions, resulting from the cleavage of chromosomes[3-5]. On the other hand, a recent study suggests that the cancer risk from CT scans is influenced by cancer-predisposing factors, such as congenital chromosomal abnormalities and immune deficiencies[6]. Furthermore, an epidemiological investigation of the atomic bomb survivors in Hiroshima and Nagasaki revealed no significant health effects due to radiation doses of 100 mSv or less[7]. Furthermore, studies analyzing the influence of chronic low-dose radiation exposure on inhabitants of high background radiation areas (HBRAs) revealed no increase in cancer and non-cancer mortality[8,9]. However, most of these studies are epidemiological investigations and not based on actual observation of the effects of radiation exposure on the human body.

The dicentric chromosome (Dic) assay (DCA), which analyzes the number of Dics formed in peripheral blood (PB) lymphocytes provides an insight into chromosomal cleavage[10]. DCA is the most sensitive biological dose assessment method with a lower limit of approximately 100 mGy for 1,000 metaphase spreads, as described in the International Atomic Energy Agency (IAEA) manual[10-12]. However, DCA has been used to assess the aberrations caused by irradiation experiments, in which the patients were exposed to radiation doses of around 100 mSv[13-16]. These reports suggest that DCA can assess radiation dose of less than 100 mSv. Conventional DCA involves the

detection of chromosomal aberrations using Giemsa staining and requires a well-trained and skilled observer. Therefore, to ease the process of detection, fluorescence in situ hybridization (FISH) could be used along with DCA[17,18]. Giemsa staining is a conventional method for DCA; however, we expect Centromere-FISH to replace Giemsa staining because it is easier to perform and inexpensive compared to telomere-centromere FISH[17,18].

Another well-known method is chromosomal translocation analysis. This method differs from DCA in it suitability for retrospective dose estimation[12]. However, dicentric chromosomes and chromosome translocations are caused by DNA double-stranded breaks in an approximately equal ratio in human PB cells after exposure to radiation[19,20]. Chromosomal translocations are an important factor in many hematologic malignancies[21]. Therefore, we reasoned that a single CT scan might produce chromosome translocations that could be the cause of leukemia or other cancer.

This study aimed for risk analysis of medical radiation exposure and establishment of an evaluation method in low dose exposure. Therefore, we performed DCA and translocation analysis using PB before and after a CT scan, which emits relatively high radiation doses compared to other low-dose radiation emitting medical procedure, to assess the effects of low-dose ionizing radiation on chromosomes.

MATERIALS AND METHODS

Subjects

The study included 12 patients (3 males and 9 females) aged 62-83 years (mean 71 years) undergoing medical examinations at the hematologic internal medicine, respiratory internal medicine, and respiratory surgery departments of Fukushima Medical University Hospital. Data regarding the history of disease and treatment, CT scans, and smoking status of the subjects are shown in table 1. Patients, who had received radiotherapy or chemotherapy previously, did not undergo any treatment a year prior to study commencement. Informed consent was obtained from all participants for analysis of the PB samples, and the experiment was performed in accordance with the guidelines approved by the Council for International Organizations of Medical Science[22]. This study was approved by the Ethics Committee of Fukushima Medical University School of Medicine (approval number 1577).

Separation of lymphocytes from PBs and cell culture conditions

PB, obtained from each patient before and after (within 3-28 days) the CT scan, was heparinized. Mononuclear blood cells were isolated using BD Vacutainer CPT tubes (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. Cells were suspended in RPMI 1640 medium (Nacalai Tesque, Kyoto, Japan) containing 20% fetal bovine serum (Equitech Bio, Keilor East, Australia), 2% phytohaemagglutinin-HA15 (Remel, Thermo Fisher Scientific, Waltham, MA, USA), and 60 μg/ml kanamycin solution (Life Technologies, Thermo Fisher Sientific) in a 15-ml Falcon tube. Lymphocytes were cultured in a 5% humidified CO_2 incubator at 37°C for 48 h. First-division metaphase cells were obtained by treatment with colcemid (final concentration, 0.05 μg/ml; Life Technologies) for 48 h.

Cell harvesting

After 48 h of culture, cells were harvested, treated with 0.075 M KCl, and fixed with methanol/acetic acid (3:1) according to the standard cytogenetic procedure[10,12]. Cell pellets were suspended in an appropriate volume of fixative as per their size. One drop (~20 µl) of the suspension was dispensed onto a pre-cleaned slide and placed on a water bath.

Giemsa staining

Each slide was immersed in 5% Giemsa (Merck Millipore, Darmstadt, Germany) solution diluted with Gurr buffer (pH6.8, Life Technologies) for 15 min and then washed with distilled water and air-dried.

Centromere-fluorescence in situ *hybridization (Centromere-FISH)*

First, each slide was dried at 65°C for an hour or more for hardening. Next, 5-6 µl of Poseidon probe (Kreatech, Amsterdam, The Netherlands) solution was applied per 22 × 22-mm area, covered with a glass coverslip and sealed with a paper bond. Nuclear DNA was denatured by incubating the slides on a hot plate at 72°C for 4 min and then in a humidified chamber overnight at 37°C for hybridization. The glass coverslips were removed and the slides were washed in post-wash buffer I (0.4 × saline-sodium citrate; SSC/0.3% Triton X-100) at 72°C for 2 min and wash buffer II (2 × SSC/0.1% Triton X-100) at room temperature (RT) for 1 min. Subsequently, the slides were dehydrated using 70% ethanol followed by 100% ethanol for 5 min and then air dried at RT. Finally, nuclei were counterstained with Vectashield Mounting Medium containing 4,6-diamidino-2-phenylindole (Vector Laboratries, Burlingame, CA, USA), covered with a glass coverslip, and sealed with nail polish.

Chromosome painting

Each slide was first dried at 65°C for at least an hour for hardening the chromosomes. Next, 6-7 µl of a Customized XCP-Mix probe (Mix-#1R-#2G-#4RG; MetaSystems,

Altlussheim, Germany) solution was added per 22 × 22-mm area, and the slide was covered with a glass coverslip and sealed with paper bond. Subsequent operations were carried out according to the manufacturer's instructions. Nuclear DNA was denatured by incubating the slides on a hot plate at 75°C for 2 min, followed by incubation overnight at 37°C in a humidified chamber to allow for hybridization. The glass coverslips were removed and the slides were washed in 0.4 × SSC at 72°C for 2 min. After draining excess SSC, the slides were then washed in 2 × SSC/0.05% Tween-20 at RT for 30 sec, immediately rinsed in distilled water to avoid crystal formation and then air dried at RT. Finally, nuclei were counterstained with Vectashield Mounting Medium containing 4,6-diamidino-2-phenylindole (Vector Laboratries), and the slides were covered with a glass coverslip and sealed with nail polish.

Image capturing and scoring of chromosomal aberrations

Soon after completing chromosome preparations, Giemsa-stained metaphase spreads and FISH images were captured in the AutoCapt mode using two sets of AXIO Imager Z2 microscopes (Carl Zeiss AG, Oberkochen, Germany) equipped with a CCD camera and Metafer 4 software (MetaSystems GmbH, Altlussheim, Germany), respectively. Metaphases for scoring were selected in manual mode. Chromosome analysis was performed according to the IAEA manual (IAEA 2001) [10] by two trained and experienced personnel with good microscopy skills. Using the selected metaphase images, all observable aberrations in 2,000 or more metaphases were scored.

Chromosomes and centromeres were enumerated, and metaphase spreads with less than 45 centromeres were omitted from analysis. We scored not only Dic, which is the most important parameter in dosimetry, but also other chromosome- or chromatid-type aberrations, such as rings, acentric fragments, and chromatid exchanges.

For translocation analysis, metaphase spreads with 44-46 chromosomes and three chromosome pairs (Chromosomes 1, 2 and 4) colored in three different paintings were selected for analysis. Metaphase cells exhibiting tetraploidy were omitted from the

analysis. Based on a previous report that indicated that almost all apparently one-way (non-reciprocal) translocations are usually actually two-way (reciprocal) translocations[23], we included apparently one-way translocations in the two-way translocation counts. In the case of complex chromosomal abnormalities, the number of translocations was determined by the number of color junctions (NCJ) [24]. An NCJ of 1 or 2 reflects one translocation, an NCJ of 3 or 4 reflects two translocations, and an NCJ of 5 or 6 reflects three translocations and so on. We also scored other chromosomal aberrations such as Dics. For scoring, the formula used to calculate the frequency of translocations across the whole genome (F_G) was based on the formula using three colors (Chromosome 1: Red, Chromosome 2: Green, and Chromosome 4: Yellow) for the detection of translocations as follows:

$$F_G = F_{P(1+2+4)} / 2.05\ [f_1(1-f_1)+f_2(1-f_2)+f_4(1-f_4) - (f_1f_2+f_1f_4+f_2f_4)]$$

F_G: the full genome aberration frequency,

F_p: the translocation frequency detected by FISH,

f_p: the fraction of genome hybridized, taking into account the gender of the subjects (female: fp = 0.2234, male: fp = 0.2271).

The proportion of the genome occupied by Chromosomes 1, 2 and 4 is ~23%. Therefore, F_G is determined by the following formula:

$$F_G = F_P \times 2.567 \text{ (female)}$$

$$F_G = F_P \times 2.533 \text{ (male)}$$

In order to combine the cell numbers of the analysis, we determined F_G, obtained according to the above formulae for females and males, as per 2000 cell equivalents.

Calculation of effective CT scan radiation dose

A Toshiba Aquilion model 64 CT scanner was used in this study, with a tube voltage of 120 kV. The effective radiation dose was calculated by inputting data regarding age, sex, and initiation and end position of the CT scan into the computational dosimetry system (WAZA-ARI: http://waza-ari.nirs.go.jp/waza_ari/login/)[25-27].

Radiation dose evaluation based on DCA results

Relationship between the number of Dics formed per cell and radiation dose is expressed by the following formula: aberrations/cell = $aD^2 + bD + c$ (where D represents the radiation dose; a represents the dose-squared coefficient; b represents the dose linear coefficient; and c represents the background frequency). Variables a, b, and c and the radiation dose were calculated on the basis of DCA data and using Chromosomal Aberration Calculation Software (CABAS: http://www.ujk.edu.pl/ibiol/cabas/)[28]. In this study, the radiation dose was estimated by comparing the results of Giemsa staining with the standard dose-response curve for DCA of the chromosome network for biodosimetry in Japan[29].

Statistical analysis

The Student's *t*-test was used to compare the number of Dics and translocations before CT scanning in patients who had or had not undergone prior treatment. The Student's paired *t*-test was used to compare the number of Dics and translocations before and after CT scanning. The relationship between increase in Dic formation and effective radiation doses was calculated by WAZA-ARI. Simple linear regression analysis was used to compare the increase in Dic formation in 1,000 and 2,000 metaphases and to compare the efficiency of detection by Giemsa staining and Centromere-FISH. Analyses were performed using STATA software, Version 11.1 (StataCorp, College Station, TX, USA). *P* values of less than 0.05 were regarded as statistically significant.

RESULTS

Subject background data

Background data pertaining to the 12 patients are shown in table 1. For the six patients with malignant lymphoma who were recruited for the study after chemotherapy (mainly rituximab plus CHOP: cyclophosphamide, doxorubicin, vincristine and prednisolone) and/or radiotherapy (treatment group), at least 5 years had elapsed between the treatments and the commencement of the present study. Two patients (Patients 3 and 7) had previously received both chemotherapy and radiotherapy. Patients who had only undergone surgery for lung cancer or CT examination for diagnosis were considered, in this study, to not have undergone prior treatment. Patients with a history of smoking had ceased smoking >10 years prior to the study. All patients had undergone chest X-ray during annual medical examinations. In addition, all patients except one (Patient 10) had undergone more than five CT scans in the past 5 years, and three malignant lymphoma patients and three lung cancer patients had undergone a positron emission tomography (PET) examination before this study. Patients with lung cancer (Patients 1 and 11) had undergone surgery, and the last patient (Patient 12) had been administered letrozole after surgery for breast cancer. With respect to medication, one patient (Patient 2) ingested 2.5 mg of predonine every other day for hay fever, four patients (Patients 3, 4, 8 and 11) were prescribed medication for hypertension, and two patients (Patients 10 and 11) were prescribed medication for diabetes. A history of smoking was believed to not to have an influence on Dic formation because of the 10-years gap since smoking cessation and study commencement.

Table 1. Patient background data

Patient No	Disease	Part of body examined in CT scan	Days from CT scan to PB collection	Treatment[#1]	Smoking status	Past CT examination[#3]	Other X-ray examinations[#4]
1	Lung cancer	Chest	8	(-)	(-)	(+)	Chest, UGI, PET
2	Lymphoma	Cervix, Chest, Abdomen, Pelvis	3	(+)	(-)	(+)	Chest, UGI
3	Lymphoma	Chest, Abdomen, Pelvis	11	(+)	(-)	(+)	Chest, UGI, PET
4	Chest abnormal shadow	Chest	15	(-)	(-)	(+)	Chest, UGI
5	Chest abnormal shadow	Chest	22	(-)	(+)[#2]	(+)	Chest, UGI
6	Lymphoma	Chest, Abdomen, Pelvis	14	(+)	(-)	(+)	Chest, UGI
7	Lymphoma	Cervix, Chest, Abdomen, Pelvis	2	(+)	(+)[#2]	(+)	Chest, UGI, PET
8	Lymphoma	Cervix, Chest, Abdomen, Pelvis	28	(+)	(-)	(+)	Chest, UGI
9	Lymphoma	Chest, Abdomen, Pelvis	7	(+)	(+)[#2]	(+)	Chest, UGI, PET
10	Chest abnormal shadow	Chest	14	(-)	(-)	(+)	Chest, UGI
11	Lung cancer	Chest	2	(-)	(+)[#2]	(+)	Chest, PET
12	Lung cancer (suspected)	Chest	6	(-)	(+)[#2]	(+)	Chest, UGI, PET

#1 Chemotherapy or radiotherapy had been performed at least five years before this study.

#2 These patients had given up smoking at least ten years before this study.

#3 All patients except one (No. 10) took examinations of CT scaning more than 5 times during the past 5 years.

#4 UGI: X-ray examination of the upper gastrointestinal tract, PET: positron emission tomography.

Analysis of Dic formation before and after CT scanning and relationship between the increase in Dic formation and dose of radiation exposure

A total of 2,000 metaphase spreads were analyzed for Dic formation by Giemsa staining and Centromere-FISH before and after a single CT scan. The number of Dics formed and estimated dose are shown in table 2. The estimated radiation dose, based on the increase in Dic formation as determined by Giemsa staining and Centromere-FISH, was compares to the standard dose-response curve and effective radiation dose of each CT scan as calculated using WAZA-ARI. A significantly higher number of Dics was observed to form after the CT scan than prior to the scan, as determined by both Giemsa staining ($p < 0.01$) and Centromere-FISH ($p < 0.01$) (figure 1). The number of Dics formed before the CT scan was compared between patients with and without a history of chemotherapy and/or radiotherapy (figure 2). A higher (but not significant) number of Dics formed in patients with a treatment history than without, as determined by both the methods. Data regarding the estimated dose in patients 4, 7, 8, and 12 in analyses using Giemsa staining and in patients 2, 4, 8, 10, and 11 in analyses using Centromere-FISH were unavailable since the increase in number of Dics formed was lower than that formed in the background of the standard dose-response curve. Even in other patients, the estimated doses obtained by using Giemsa staining, Centromere-FISH, and WAZA – ARI did not correspond with each other.

Table 2A. Increase in Dic formation after a single CT scan (Giemsa staining)

Patient No	Number of Dics/2000 metaphases		Increment	Estimated dose (mSv) by DCA	WAZA-ARI (mSv)
	Before CT	After CT			
1	5	8	3/2000	2.34	5.78
2	5	9	4/2000	14.76	21.90
3	9	12	3/2000	2.34	23.26
4	5	7	2/2000	ND#	6.85
5	0	4	4/2000	14.76	12.99
6	3	10	7/2000	49.68	23.07
7	15	17	2/2000	ND	23.21
8	12	14	2/2000	ND	60.27
9	4	10	6/2000	38.39	40.96
10	1	1	0/2000	0.00	24.13
11	5	5	0/2000	0.00	20.66
12	4	6	2/2000	ND	37.30

ND = Not detected

Table 2B. Increase in Dic formation after a single CT scan (Centromere-FISH)

Patient No	Number of Dics /2000 metaphases		Increment	Estimated dose (mSv) by DCA	WAZA-ARI (mSv)
	Before CT	After CT			
1	2	5	3/2000	2.34	5.78
2	3	5	2/2000	ND	21.90
3	5	9	4/2000	14.76	23.26
4	3	4	1/2000	ND	6.85
5	3	6	3/2000	2.34	12.99
6	2	8	6/2000	38.39	23.07
7	11	15	4/2000	14.76	23.21
8	13	14	1/2000	ND	60.27
9	2	8	6/2000	38.39	40.96
10	0	1	1/2000	ND	24.13
11	7	8	1/2000	ND	20.66
12	3	6	3/2000	2.34	37.30

ND = Not detected

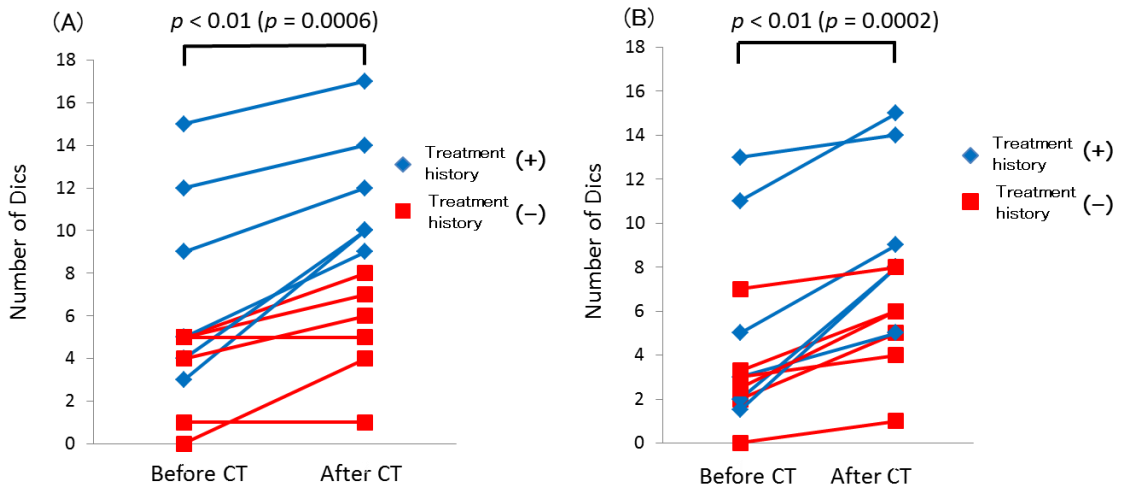

Figure 1. Comparison of the number of Dics formed before and after the CT scan. Significantly more Dics were formed after the CT scan than before the CT scan, as determined using both Giemsa staining (p = 0.0006) (A) and Centromere-FISH (p = 0.0002) (B).

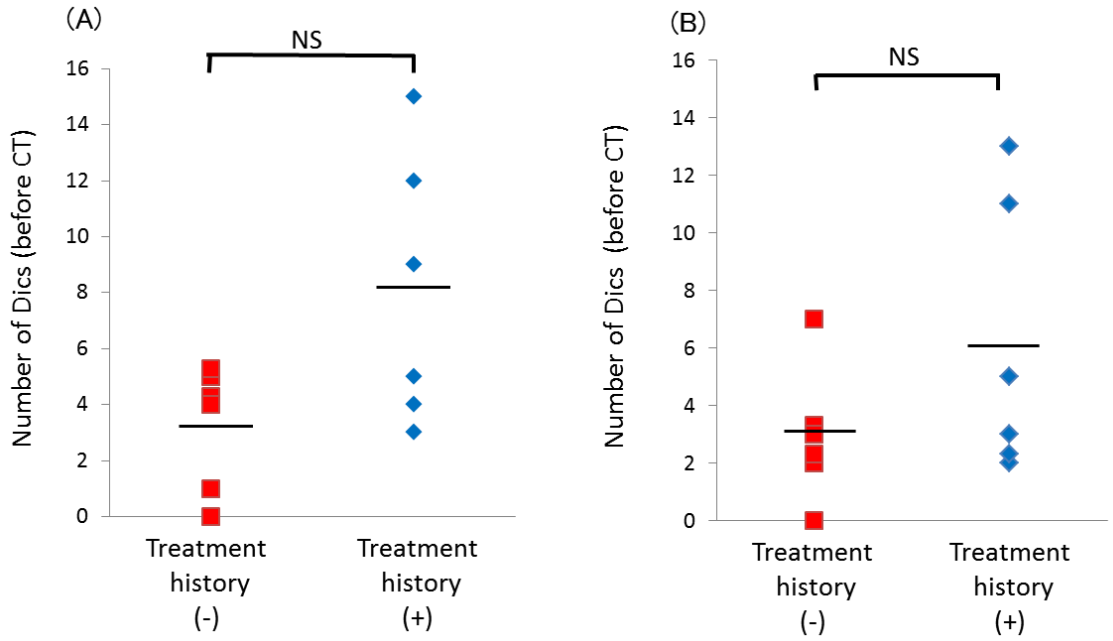

Figure 2. Number of Dics formed before the CT scan in patients with or without treatment history. There was no significant difference between patients with and without treatment history, as determined using both Giemsa staining (A) and Centromere-FISH (B). NS: no significant difference.

Comparison of the frequency of chromosome translocation before and after CT scanning

To compare the number of cells analyzed in Dic and translocation analyses, we analyzed 5000 metaphases, which was equivalent to the whole-genome analysis of almost 2000 metaphase spreads (table 3). We compared the frequency of chromosome translocations before the CT scan for patients with and/or without a history of chemotherapy and/or radiotherapy and found that the frequency of chromosome translocation tended to be higher in patients with previous treatment; but this difference was not significant. Notably, the frequency of translocations detected after the CT scan was not significantly higher than that detected before the CT scan (figure 3). Therefore,

the frequency of chromosome translocations compared to Dic formation, did not show the same tendency to increase after a CT scan.

Table 3. Increase in Chromosome translocation after a single CT scan (chromosome 1, 2, 4-painting)

Patient No.	Timing	Number of cells scored		Number of translocations	Increment	Frequency of observed translocation[#2]
		Cell count of analysis	Cell equivalent[#1]			
1	Before CT	5131	2011	43	7	2.14
	After CT	5126	2009	50		2.49
2	Before CT	5184	2031	172	29	8.47
	After CT	5134	2012	201		9.99
3	Before CT	5128	2010	3809	128	189.50
	After CT	5139	2014	3937		195.48
4	Before CT	5126	2009	26	13	1.29
	After CT	5106	2001	39		1.95
5	Before CT	5118	2006	21	12	1.05
	After CT	5138	2014	33		1.64
6	Before CT	5120	2007	671	-40	33.43
	After CT	5135	2012	631		31.36
7	Before CT	5133	2012	1067	82	53.03
	After CT	5156	2021	1149		56.85
8	Before CT	5121	2007	215	-7	10.71
	After CT	5134	2012	208		10.34
9	Before CT	5122	2008	85	12	4.23
	After CT	5104	2001	97		4.85
10	Before CT	5139	2014	37	60	1.84
	After CT	5128	2010	97		4.83
11	Before CT	5134	2012	65	19	3.23
	After CT	5111	2003	84		4.19
12	Before CT	5137	2014	43	-11	2.14
	After CT	5120	2007	32		1.59

#1 = Cell count * 196/500 (The formula is written in material and methods)
#2 per 100 cells equivalent

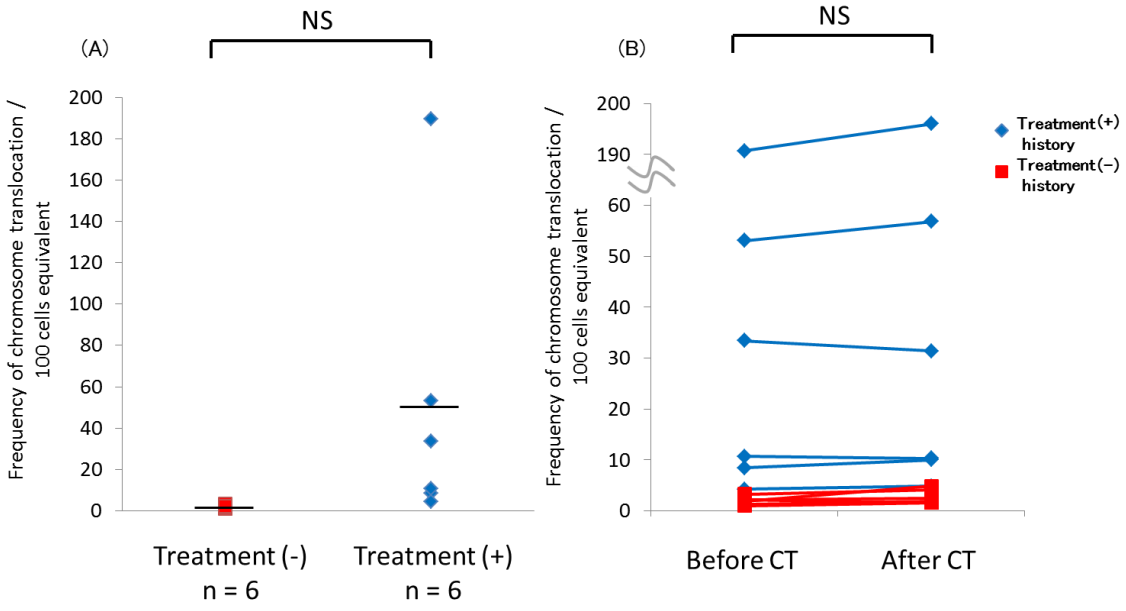

Figure 3. Comparison of the frequency of translocations of patients examined CT scan. There was no significant difference of the translocation frequencies between patients with and without treatment history (A). No significant difference was found between the frequency of translocations before and after the CT scan in patients (B). NS: no significant difference.

Comparison of Dic analyses of 1,000 and 2,000 metaphases using Giemsa staining and/or Centromere-FISH

Although the IAEA recommends the analysis of 1,000 metaphase spreads for biodosimetry using DCA at the time of a radiation exposure emergency[3)], the number of cells that must be analyzed to detect Dic formation following exposure to low-dose radiation (less than 100 mSv) need to be increased. Here, we analyzed 2,000 metaphases in a patient and assessed the reliability of results obtained from analyses of 1,000 and 2,000 metaphases using both methods. The number of Dics formed before and after the CT scan, determined using Giemsa staining or Centromere-FISH in 1,000 metaphase spreads is shown in table 4. Results of the analyses of 1,000 and 2,000 metaphases spreads using Centromere-FISH staining revealed a good correlation

between them in the increase in Dic formation, while results using Giemsa staining were not correlated (Fig. 4). Furthermore, results of the increase in Dic formation using Giemsa staining and those using Centromere-FISH were correlated in the analysis of 2,000 metaphase spreads but not in the analysis of 1,000 metaphase spreads (Fig.5).

Table 4A. Increase in Dic formation after a single CT scan (Giemsa staining/1000 metaphases)

Patient No	Number of Dics/1000 metaphases		Increment	WAZA-ARI (mSv)
	Before CT	After CT		
1	3	5	2/1000	5.78
2	5	5	0/1000	21.90
3	7	11	4/1000	23.26
4	4	4	0/1000	6.85
5	0	2	2/1000	12.99
6	2	4	2/1000	23.07
7	9	11	2/1000	23.21
8	5	9	4/1000	60.27
9	2	5	3/1000	40.96
10	1	0	-1/1000	24.13
11	3	4	1/1000	20.66
12	2	3	1/1000	37.30

Table 4B. Increase in Dic formation after a single CT scan (Centromere-FISH/1000 metaphases)

Patient No	Number of Dics/1000 metaphases		Increment	WAZA-ARI (mSv)
	Before CT	After CT		
1	2	2	0/1000	5.78
2	1	2	1/1000	21.90
3	2	5	3/1000	23.26
4	2	2	0/1000	6.85
5	2	2	0/1000	12.99
6	1	5	4/1000	23.07
7	5	7	2/1000	23.21
8	7	8	1/1000	60.27
9	1	4	3/1000	40.96
10	0	1	1/1000	24.13
11	5	2	-3/1000	20.66
12	2	3	1/1000	37.30

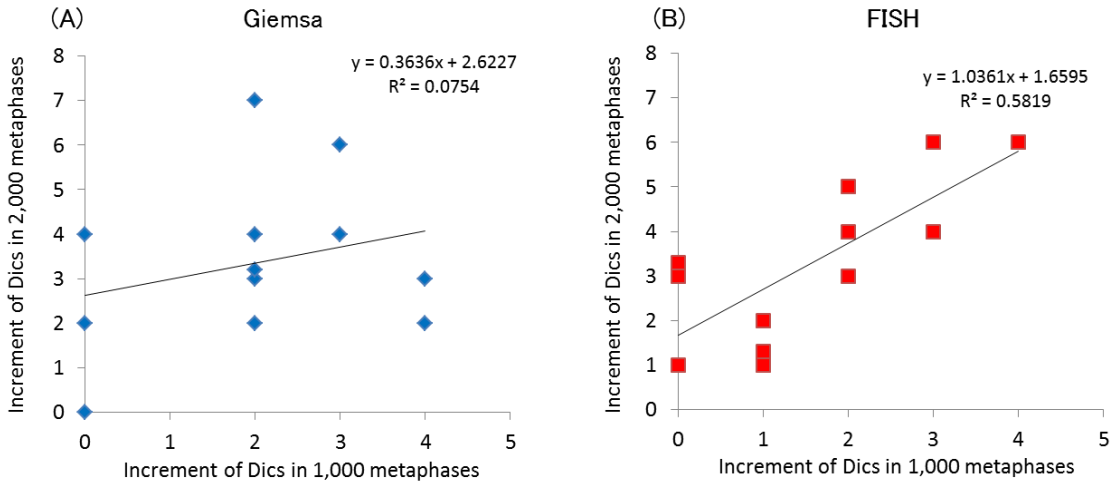

Figure 4. Relationship of the increment of Dic formation between analysis of 1,000 and 2,000 metaphases using either Giemsa staining or Centromere-FISH. No correlation was observed with the results of Giemsa staining (R^2 = 0.06692) (A), but a correlation was observed with the results of Centromere-FISH (R^2 = 0.62474) (B).

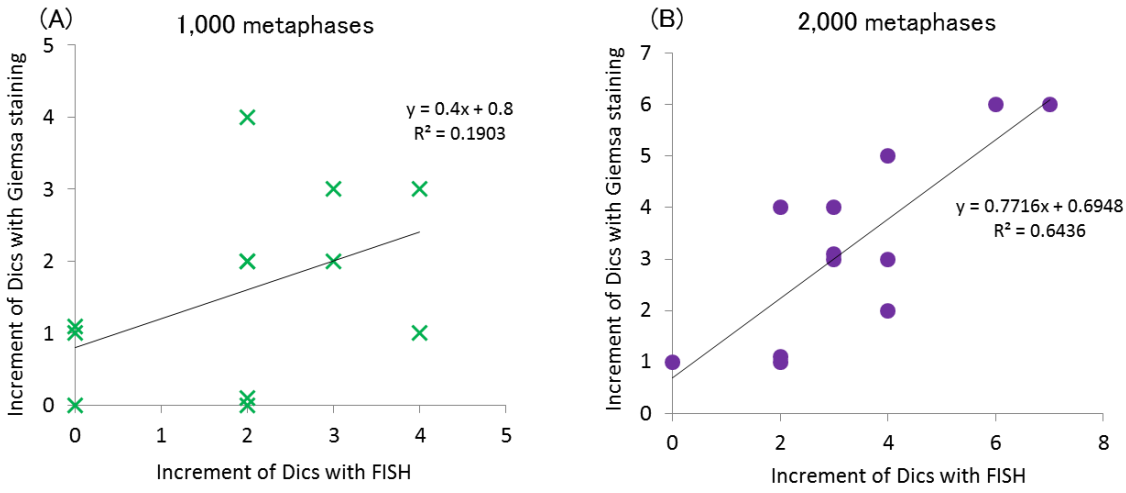

Figure 5. Relationship of the increment of Dic formation between the results of Giemsa staining and Centromere-FISH in analysis of either 1,000 or 2,000 metaphases. No correlation was observed in the analysis of 1,000 metaphase spreads (R^2 = 0.18686) (A), but a correlation was observed in the analysis of 2,000 metaphase spreads (R^2 = 0.65712) (B).

Relationship between the sample area for CT scanning and chromosomal abnormality

Moreover, we compared the relationship between the sample areas for CT scanning and induced chromosomal aberrations. Parts of the body examined in the CT scan and effective radiation dose as calculated using WAZA-ARI, are shown in table 5. A steep slope was observed on the graph when the area under the CT scan is large (Fig. 6). However, the estimated dose is inconsistent with the sample area for the CT scan.

Table 5. CT scan part and estimate dose

Patient No	Part of body examined in CT scan	WAZA-ARI (mSv)
2	Cervix, Chest, Abdomen, Pelvis	21.9
7	Cervix, Chest, Abdomen, Pelvis	23.21
8	Cervix, Chest, Abdomen, Pelvis	60.27
3	Chest, Abdomen, Pelvis	23.26
6	Chest, Abdomen, Pelvis	23.07
9	Chest, Abdomen, Pelvis	40.96
1	Chest	5.78
4	Chest	6.85
5	Chest	12.99
10	Chest	24.13
11	Chest	20.66
12	Chest	37.3

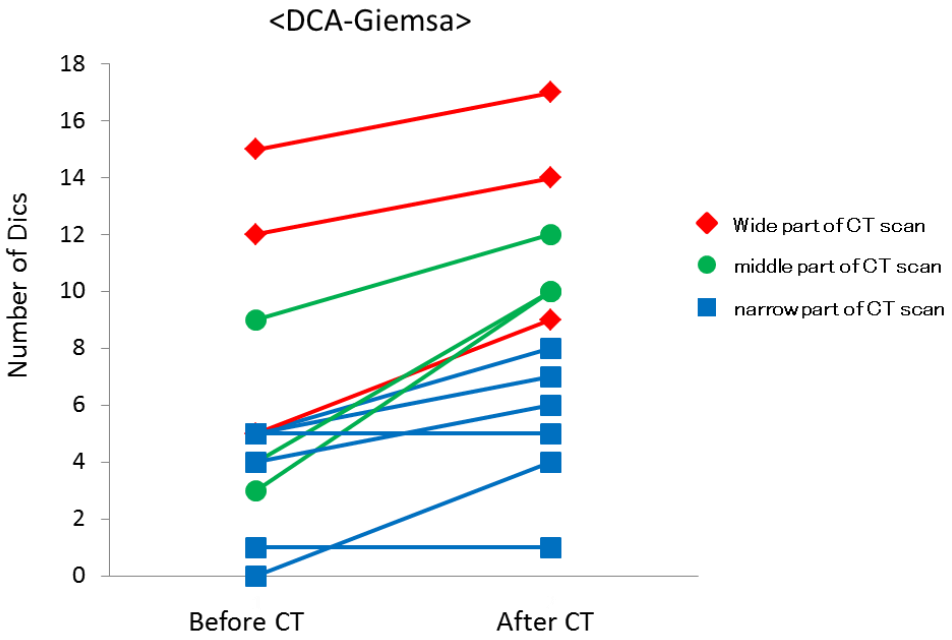

Figure 6. The relation between increment of Dic formation and CT scanning part. This shows trend of steep slope on a graph when CT scanning part is wide. But, estimate dose is not consistent with the CT scan part.

DISCUSSION

In this study, we performed DCA and translocation analysis in adult PB lymphocytes to determine the occurrence of chromosome aberrations in response to exposure to low-dose radiation (i.e., less than 100 mSv). Whole body CT scan results in the exposure to radiation doses of around 50 mSv. Detection of radiation exposure due to CT scanning seems to be difficult considering the detection lower limit of DCA. In this study, the frequency of Dics were observed to have increased in all patients after a single CT scan examination, as determined by both Giemsa staining (p = 0.0006) and centromere-FISH (p = 0.0002). These results suggest that DCA can be used to evaluate low-dose radiation exposure (less than 100 mSv), and chromosome cleavage may be induced by one CT scan. DCA is the gold standard method of biological dosimetry[11,12]; however, accurate calculation of the radiation exposure dose is difficult under presently established analysis conditions. Only lymphocytes localized in the tissues being scanned are exposed to ionizing radiation caused by CT scanning, which then are diluted in the blood and distributed equally throughout the body via blood circulation. These lymphocytes with Dics usually die within several months or the double stranded breaks are repaired, indicating that the Dics formed in PB lymphocytes are lower than the actual number of Dics induced by exposure to ionizing radiation. Therefore, blood collection within a month of the CT scan is recommended[12]. In this study, blood was collected within 3-28 days of the CT scan. Considering these factors, it is notable that we found an increase in Dic formation in all 12 patients after only one CT scan.

We observed an increase in the number of Dics by both Giemsa staining and Centromere-FISH following CT scanning. Patients who had previously undergone radiotherapy and/or chemotherapy were expected to show a higher increase in Dics than that by other patients. Five years since treatment, patients still tended to have a large number of chromosomal abnormalities before the CT scan, especially those who had received radiotherapy and chemotherapy (Patients 3 and 7). However, the number of

chromosome aberrations before the CT scan was not statistically significant, and the increase in Dics was to the same degree as that seen in patients without a treatment history. The non-significance of results can be attributed to the small sample size. And a similar trend was also observed in translocation analysis. Unstable chromosomal abnormalities such as Dic are not retained for more than a few months[12, 22-25], while the number of Dics formed reportedly increases over time in persons living in HBRAs[26,27]. However, an increase in the cancer incidence in HBRAs has not been observed[7,8]. Radiotherapy and chemotherapy are extremely powerful tools in cancer treatment and, likely, induce chromosomal abnormalities from its action mechanism[28,29]. Chemotherapy and radiotherapy have been known to induce the occurrence of a second cancer. However, these cancers are not considered to be chromosomal translocations induced by high-dose radiation exposure due to somatic mutations induced by low-dose radiation exposure. Therefore, it cannot be said that the Dics that are observed before and after the CT scan will immediately lead to the occurrence of diseases such as cancers.

A specific chromosomal translocation is observed in some cancers and blood tumors and so on. There is concern that CT scans are related to increased cancer risk in children and young adults[1,2]. Therefore, we analyzed the occurrence of chromosome translocations, which are suspected to be involved in the development of cancer. Chromosome translocations were also expected to increase significantly, resulting from the significant increase in Dic formation after CT scan. However, the frequency of chromosome translocations was higher, but not significantly so, than the number of Dics formed, both before and after the CT scans. This increase in the frequency of translocations could be attributed to reasons such as aging and smoking[30-36]. Therefore, these results suggest that chromosome translocation has lower radiation specificity than Dic. Additionally, although the cause is unknown, it is difficult to interpret the results obtained from translocation analysis and say that translocation is more frequently caused compared to Dic[37,38]. Cho *et al*. reported that the *in vivo* half-life of reciprocal

translocation in stable cells was beyond the normal life expectancy[33]. Cells with chromosome translocations are mitotically stable and able to pass through repeated cell division, and therefore, the frequency of these translocations is thought to reflect the accumulation of translocations due to exposure to chemicals or cigarette smoking over a long period, or medical radiation exposure in the treatment of various diseases. Therefore, the increase in chromosome translocations induced by a CT scan could be concealed with the frequency of chromosome translocations due to the mentioned factors. Accumulation of chromosomal aberrations in HBRA is found to be correlated with the cumulative dose, and high levels of natural radiation in HBRA have been found to be unassociated with increased cancer risk[39,40]. In any case, no significant additional increase in chromosome translocations was detected, suggesting that the contribution of radiation exposure by CT scan to carcinogenesis is small.

The analysis of 1,000 metaphase spreads by DCA is recommended in cases of exposure to γ-ray radiation doses of around 100 mSv[12], which can be reduced to approximately 70 mSv by analyzing around 10,000 metaphase spreads[11]. Here, we analyzed 2,000 metaphase spreads because analysis of 10,000 would have been very difficult. Suto *et al*. performed DCA of 1,000 metaphases by Giemsa staining of PB lymphocytes of workers mean to engage in emergency response tasks at the Fukushima Daiichi Nuclear Power Station and compared their results with radiation exposure recorded using personal physical dosimeters. They found a correlation between DCA results and personal dosimeter records with respect to radiation doses in the range of 26-171 mSv[16]. Iwasaki *et al*. demonstrated a linear dose response with respect to chromosome aberrations in human lymphocytes exposed to less than 50 mSv of γ-rays in an analysis of more than 5,000 metaphase spreads using a semi-automated metaphase-finding/relocation system[41]. These studies confirm that DCA is useful for evaluating exposure to radiation doses of less than 100 mSv, although the analysis of more than 2,000 metaphase spreads may be necessary. Some previous studies have reported that FISH is more accurate than conventional Giemsa staining for dose

estimation[17,18]. In agreement with these studies, we also recommend the use of FISH for DCA in cases of low-dose radiation exposure because in contrast to results obtained for Giemsa staining, we found a good correlation between the results of analyses of 1,000 and 2,000 metaphase spreads using Centromere-FISH in this study. Therefore, it is recommended that the analysis of 2,000 metaphase spreads or more be performed by Giemsa staining and that of 1,000 metaphase spreads be performed by Centromere-FISH to achieve equivalent levels of precision.

The CT examination range is considered a factor that affects the induction of chromosomal abnormality. Therefore, we compared the relationship between CT scanning and induced chromosomal aberrations. It was observed that scanning of larger sample areas led to increasing Dic after the scan. However, the result was not statistically significant, and the effective doses calculated using WAZA-ARI were also insignificant. This is thought to be CT scan conditions are different, such as scan, probably due to differing CT scanning conditions of number/scan area. The wider the CT scan range, greater are the number of cells exposed. In other words, it is suggested that chromosomal abnormality may be induced in exposed cells. In order to consider this causal relationship, analysis of more samples is necessary.

The dose of radiation exposure associated with a CT scan is relatively high for medical radiation-related exposure. An increase in the risk of cancer in children and young adults because of CT scanning is a cause for concern[1,2]. However, at present, there are no published studies comparing the frequency of chromosomal abnormalities such as Dic and translocation before and after a CT scan. This is, to our knowledge, the first report on such a comparison. In this study, we found that Dic formation significantly increased in PB lymphocytes of adults after a CT scan, while in the same patients, no significant increase was observed in the frequency of chromosome translocations. The effect of continued radiation exposure using CT scan and differences in radiosensitivity between children and adults could influence these results. Therefore, we conclude that DCA is useful for evaluating radiation exposure due to CT scanning

(less than 100 mSv), but analysis of 2,000 metaphase spreads or more by Giemsa staining or centromere-FISH is necessary. Additionally, the study results suggest that a single CT scan could hardly cause an increase in the risk of carcinogenesis.

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ABSTRACT

「CT スキャンにより誘発されるヒト染色体異常の解析」

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CT は非常に優れた診断機器であり、日本は世界の中でも有数の CT 保有大国である。CT 検査によって生じる被ばく線量はごくわずかなものであるが、低線量被ばくの健康影響に関する報告は年々増加しており、小児期における CT 検査で白血病などの発がんリスクが増加するといった報告もある。低線量の被ばくに関しては、生物学的線量評価法のゴールドスタンダードである二動原体染色体（dicentric chromosome: Dic）解析（dicentric chromosome assay: DCA）による被ばく量評価の報告もある。我々は低線量医療被ばくの中でも比較的線量の高い CT 検査（数 mGy～数十 mGy）による染色体異常頻度の変化について、ギムザ染色および FISH 法による DCA およびペインティング法による 1 番、2 番、4 番染色体の転座解析を行った。また、低線量被ばくにおける各解析方法の有用性についても検討した。

対象者は福島県立医大を受診した 62～83 歳までの男女 12 名（男性 3 名、女性 9 名、平均 71 歳）。CT 前後の末梢血リンパ球から作製した染色体標本を各解析方法(ギムザ法、Centromere-FISH 法、ペインティング法)につき 2,000 メタフェース以上解析した。

結果、ギムザ法、Centromere-FISH 法ともに 1 回の CT 後に Dic 数の有意な増加が認められた。一方で転座解析では Dic 数よりも多くの転座が認められたが 1 回の CT 前後では有意な転座数の増加は認められなかった。また各解析法ともに過去に放射線治療・化学療法を行った群と行っていない群に分けて比較したが、CT 前の時点での染色体異常頻度に有意な差は認められなかった。また DCA では 2,000 メタフェースの解析で両解析法の Dic 増加量が相関し、Centromere-FISH 法では 1,000 メタフェ

ースでも評価ができる可能性が示唆された。一方、Dic 増加量と推定線量間では相関が認められず、現状ではDCAによる100 mGy未満の放射線被ばくによる染色体異常の検出は可能だが、定量的な評価は難しいことが示唆された。

本研究結果から、1 回の CT スキャンによって染色体切断が誘発されることが示唆された。しかし、染色体転座の有意な増加は認められなかった。この点から、CT スキャンによる放射線被ばくの発がんへの寄与は小さいものと考えられる。また、CT 検査範囲は染色体異常の誘発に影響を及ぼす要因の 1 つとして考えられたが、本研究ではサンプル数が少ないことからCTスキャンによる染色体異常誘発因子として同定することはできなかった。本研究結果は、DCAがCT検査(100 mSv未満)による放射線被ばくを評価するのに有用であることを示唆するものであるが、ギムザ法、Centromere-FISH 法のどちらの解析法においても 2,000 メタフェース以上の解析が必要である。