Investigation of the cumulative number of chromosome aberrations induced by three consecutive CT examinations in eight patients

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ABSTRACT

In our previous study, we found that chromosomes were damaged by the radiation exposure from a single computed tomography (CT) examination, based on an increased number of dicentric chromosomes (Dics) formed in peripheral blood lymphocytes after a CT examination. We then investigated whether a cumulative increase in the frequency of Dics and chromosome translocations (Trs) formation could be observed during three consecutive CT examinations performed over the course of 3–4 years, using lymphocytes in peripheral bloods of eight patients (five males and three females; age range 27–77 years; mean age, 64 years). The effective radiation dose per CT examination estimated from the computational dosimetry system was 22.0–73.5 mSv, and the average dose per case was 40.5 mSv. The frequency of Dics formation significantly increased after a CT examination and tended to decrease before the next examination. Unlike Dics analysis, we found no significant increase in the frequency of Trs formation before and after the CT examination, and we observed no tendency for the frequency to decrease before the next CT examination. The frequency of Trs formation was higher than that of Dics formation regardless of CT examination. Furthermore, neither analysis of Dics nor Trs showed a cumulative increase in the frequency of formation following three consecutive CT examinations.

Keywords: chromosome aberration; dicentric chromosome; chromosome translocation; computed tomography; effective radiation dose

INTRODUCTION

The annual radiation exposure dose of the Japanese population is about 2.1 mSv, which is derived from natural sources, plus about 3.9 mSv, which is derived from artificial ionizing radiation. Almost all of the latter is due to medical radiation exposure, and the majority of it is due to computed tomography (CT) examination [1, 2]. CT examination is a very useful diagnostic method and its use has increased rapidly. However, the radiation exposure dose of abdominal CT examination in an adult is around 20 mSv, and the relationship between radiation exposure from frequent CT examinations and the prevalence of cancer is a concern [3, 4]. Pearce et al. reported that CT examination is a risk factor for development of leukemia and brain tumors in children.
Furthermore, the prevalence of cancer is higher in a group that underwent CT examination compared with a group that did not, according to a cohort study of 11 million children and adolescents [6]. On the other hand, after excluding children with congenital chromosomal aberrations and immunodeficiency such as HIV infection, which are cancer-predisposing factors, no significant increase in the prevalence of cancer was observed [7]. In previous studies, we analyzed dicentric chromosomes (Dics) formation and chromosome translocations (Trs) to determine whether such Dics and Trs are induced by ionizing radiation exposure due to CT examination [8, 9]. We showed an increase in Dics formation after a single CT scan (5.57–60.27 mSv: mean 24.24 mSv), but the frequency of Trs detected before and after the CT scan did not differ significantly. Therefore, the data suggested that DNA double-strand breaks (DSBs) could be induced by the radiation exposure from one CT scan. However, an increase in Trs was not likely to be detected because of the high baseline due to multiple confounding factors in adults. We here investigated whether cumulative Dics and Trs formation could be observed during three consecutive CT examinations performed over the course of 3–4 years, using lymphocytes in peripheral blood (PB).

MATERIALS AND METHODS

Ethics statement
The samples and the medical records used in our study have been approved by the Ethics Committee of the Fukushima Medical University School of Medicine (approval number 1577). Written informed consent was obtained from all participants for analysis of PB samples, and the methods were carried out in accordance with approved guidelines of the Council for International Organizations of Medical Science [10].

Blood donors
PB samples were collected from eight patients (five male and three female) aged 27–77 years (mean 64 years) who were followed in the Department of Hematological Internal Medicine at Fukushima Medical University Hospital. A total of six samples were collected from the same patient for analyses before and after three consecutive CT examinations. The follow-up was approximately 1 year after each CT examination.

Lymphocyte culture
Blood samples were taken just before and within a month after each CT examination (Table 1). Mononuclear blood cells were isolated from heparinized PB from each sample 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, Lenexa, KS, USA) and 60 μg/ml kanamycin solution (Life Technologies, Carlsbad, CA, USA) in a tube. Lymphocytes were cultured in a 5% humidified CO2 incubator at 37°C for 46 h. Then, colcemid solution (Wako, Osaka, Japan) was added (final concentration: 50 ng/ml or 0.05 μg/ml) and cells were cultured for an additional 2 h. After 48 h of culture, chromosome preparations were made according to the standard cytogenetic procedure [11].

Chromosomes on each slide were stained with two methods. Centromere-fluorescence in situ hybridization (Centromere-FISH) was performed with the Poseidon probe (KRATECH, Amsterdam, The Netherlands) according to the manufacturer’s protocol with slight modifications as described in our previous study [8]. Chromosome painting was performed with the Customized XCP-Mix probe for chromosomes 1, 2 and 4 (Mix-#1R+2G+4RG; MetaSystems, Altlussheim, Germany) according to the manufacturer’s protocol.

Image capturing and scoring
FISH images were captured in the AutoCapt mode using two sets of AXIO Imager Z2 microscopes (Carl Zeiss AG, Oberkochen, Germany) equipped with CCD cameras and Metafer 4 software (MetaSystems GmbH). In total, more than 2000 metaphase chromosomes were scored in the Centromere-FISH slide [8]. Chromosomes were classified as dicentric or multicentrometric (chromosomes with three or more centromeres). Metaphase chromosomes with <45 centromeres were omitted from analysis. For the Trs analysis, >5000 metaphase chromosomes were scored in each sample [9]. Based on a previous report [13], we included apparently one-way Trs in the two-way Trs counts. In the case of complex chromosomal abnormalities, the numbers of Trs were determined based on the number of color junctions [14]. Other chromosome- or chromatid-type aberrations were also recorded such as rings, acentric fragments, breaks and gaps. For scoring, the formula used to calculate the frequency of Trs formation across the whole genome (FG) was based on the formula using three colors (chromosome 1: red, chromosome 2: green, chromosome 4: yellow) for the detection of translocations as follows [12]:

\[ F_G = \frac{F_T (1 + 2 + 4)}{2.05 \times \left[ f_1 (1-f_1)+f_2 (1-f_2)+f_4 (1-f_4)-f_1 f_2-f_1 f_4-f_2 f_4 \right] + f_3 f_4} \]

\[ F_G : \] the whole genome aberration frequency,

\[ F_T : \] the translocation frequency detected by FISH,

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

The proportion of the genome occupied by chromosomes 1, 2 and 4 is about 23%. Therefore, \( F_G \) is determined by the following formula:

\[ F_G = F_T \times 2.567 \] (Female)
\[ F_G = F_T \times 2.533 \] (Male)

To unify the cell numbers for the analysis, we determined \( F_G \) per 2000 cell equivalents, which were obtained according to the above formulas for females or males. For FISH slides, we switched to each captured filter image and carefully checked for the presence or absence of a Trs signal.

Calculation of the 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 the initiation and end position of
Table 1. Patient characteristics

| Patient No | Sex | Age | Disease | Part of body examined in CT scan | Period between 1st and 2nd (days) | Period between 2nd and 3rd (days) | Days from CT examination to PB collection (1st/2nd/3rd) | DLP (mGy cm) | WAZA-ARI (mSv) (1st/2nd/3rd) | Treatment | Smoking status | Part CT examination | Part other X-ray examinations |
|------------|-----|-----|---------|---------------------------------|----------------------------------|----------------------------------|---------------------------------------------------|-------------|---------------------------|------------|----------------|---------------------|--------------------------------|
| 1          | Male | 63  | Lymphoma | Cervix, chest, abdomen, pelvis  | 391                              | 380                              | 8/16/7                                            | 3679.70/3695.85/3291.20 | 46.30/57.88/57.17 | (+) Chemo | (−)               | (+)                 | Chest, UGI, PET              |
| 2          | Female | 67 | Lymphoma | Cervix, chest, abdomen, pelvis  | 331                              | 350                              | 9/19/7                                            | 1682.30/4354.12/4222.08 | 30.82/75.22/68.25 | (−)       | (−)               | (+)                 | Chest, UGI, PET              |
| 3          | Male | 64  | Abnormal chest shadow | Cervix, chest, abdomen, pelvis | 364                              | 364                              | 7/7/7                                             | 5856.00/4119.80/3321.70 | 54.40/69.47/60.16 | (−)       | (−)               | (+)                 | Chest, UGI                      |
| 4          | Male | 51  | Lymphoma | Cervix, chest, abdomen, pelvis  | 341                              | 352                              | 6/9/7                                             | 3319.80/3046.10/2635.20 | 61.83/50.67/46.72 | (−)       | (−)               | (+)                 | Chest, UGI                      |
| 5          | Male | 73  | Lymphoma | Chest, abdomen, pelvis          | 490                              | 364                              | 7/14/14                                           | 2488.40/1990.50/1704.20 | 46.22/37.25/32.46 | (+) Chemo | (−)               | (−)                 | Chest, UGI, PET              |
| 6          | Female | 27| Lymphoma | Cervix, chest, abdomen, pelvis  | 153                              | 358                              | 6/15/14                                           | 1248.00/2039.93/1656.50 | 27.06/35.95/33.73 | (+) Chemo | (−)               | (−)                 | Chest, PET                     |
| 7          | Male | 77  | CML      | Chest, abdomen, pelvis          | 445                              | 280                              | 11/14/31                                          | 3368.50/3064.30/2405.70 | 62.61/56.59/46.98 | (+) TKI   | (+)               | (+)                 | Chest, UGI                      |
| 8          | Female | 63| Lymphoma | Cervix, chest, abdomen, pelvis  | 742                              | 700                              | 14/7/7                                            | 3265.60/3113.00/2737.00 | 63.54/64.21/56.96 | (+) Chemo, RT | (−)               | (−)                 | Chest, UGI                      |

*aCTDI was defined using 16 cm phantom. All other CTDI were defined using 32 cm phantom.

*b Each effective radiation dose of the CT scan was calculated by the computational dosimetry system (WAZA-ARI).

*c Chemotherapy or radiotherapy had been performed at least 3 years before this study. Chemo = chemotherapy (mainly rituximab plus CHOP = cyclophosphamide, doxorubicin, vincristine and prednisolone); TKI = tyrosine kinase inhibitor; RT = radiotherapy

*d Patients 2, 4 and 7 had stopped smoking 1 year before this study. Patient 3 was still smoking at the beginning of the study.

*e All patients underwent CT scanning more than five times during the past 5 years.

*f Treatment with tumor resection without additional chemotherapy or radiotherapy.
the CT scan into the computational dosimetry system (WAZA-ARI: http://waza-ari.nirs.go.jp/waza_ari/) [15–17]. Dose-length products (DLP) were extracted from parameters of the CT scan.

### Statistical analyses

The differences in the Dics and Trs frequency between before and after a CT examination were assessed using the Student’s paired t-test. A trend in the Dics and Trs frequency due to an ~1-year interval was also assessed. A P-value of <0.05 was considered significant. Multiple comparisons by Bonferroni’s method were performed to evaluate accumulation of chromosomal abnormalities by continuous CT examination. Specifically, the ‘adjusted significance level ($\alpha’)$′ was obtained by the Bonferroni method, and the probability value of the Student’s paired t-test result of each comparison pair was judged by $\alpha’ (0.017)$. Analyses were performed using IBM SPSS Statistics 25 (IBM Corp. Armonk, NY, USA).

### RESULTS

#### Patient background data

Patient background data are shown in Table 1. Eight patients were assessed, because not many patients remained in remission after chemotherapy and/or radiotherapy, and were followed by CT examination. The eight patients in this study were different from those in a previous study [8, 9]; five patients with malignant lymphoma (ML) were in remission and had not received treatment for more than 3 years after the end of treatment, one patient with ML received tumor resection treatment only, one patient had been followed due to an abnormal lung shadow without treatment, and another one patient with chronic myelogenous leukemia (CML) was receiving treatment with a tyrosine kinase inhibitor (TKI) and showed a major molecular response. This patient underwent consecutive CT examinations for the follow-up after resection of colorectal cancer. Four patients were smokers. CT examination was performed once a year except for patient 8 whose CT examination interval was about 2 years. All patients did not take upper gastrointestinal tract (UGI) examination or positron emission tomography (PET) examination during this research period. The effective radiation dose per CT examination estimated from the computational dosimetry system (WAZA—ARI) was 27.1–75.2 mSv, and the average dose per patient was 51.6 mSv (Tables 1 and 2).

#### Frequency of Dics formation and effective radiation dose per CT examination

Actual analyzed cell number and the distribution of Trs number are shown in Supplementary Table 1 (see online Supplementary material). The frequency of Trs formation and effective dose per CT examination are shown in Table 2. To calculate the frequency of Trs formation in all chromosomes from those of chromosomes 1, 2 and 4, 5000 cells were analyzed, which corresponds to 2000 cells as in Dics analysis because the DNA content of chromosomes 1, 2 and 4 accounts for 23% of the DNA content in all chromosomes [12]. The frequency of Trs formation on all chromosomes, expressed as the frequency per 100 cells, and the increase/decrease in the frequency of Trs formation before and after each CT examination were determined. Changes in the number of Trs formation converted into the equivalent per 100 cells are shown in Fig. 2A. Unlike Dics analysis, we observed no significant increase in the frequency of Trs formation before and after the CT examination (Fig. 2B), and we found no accumulations of Trs formation after three consecutive CT examinations (Fig. 2C). The frequency of Trs formation was higher than that of Dics formation regardless of CT examination. We suspect that a cause of the high frequency of Trs formation in patient 8 regardless of CT examination (Fig. 2A) was the influence of the combination of chemotherapy and radiotherapy for lymphoma.

We also analyzed the relationship between the increment of chromosomal aberrations frequency and smoking status (Supplementary Table 2, see online Supplementary material) with reference to the research by Zhang et al. [18]. Although the number of samples was small, we could not find a significant relationship between them.

Therefore, a noteworthy point in this study is that a cumulative increase in the frequency of Dics and Trs formation after three consecutive CT examinations was not observed in the eight patients studied; the chromosomes in these patients may have been affected by aging, treatment for their disease and smoking.

#### DISCUSSION

The important point of this study is that we analyzed whether Dics and Trs formation increase cumulatively with multiple CT examinations in which the radiation exposure dose per CT examination is < 100 mSv. Although the number of analyzed cases was small, the analyzed 2000 genomes per sample for both Dics and Trs analysis was twice the number of analyses in the standard method.

In our previous study, we indirectly found that chromosomes are damaged by radiation exposure from a single CT examination, as shown by an increase in the frequency of Dics formation after CT examination [8]. However, we did not detect a significant increase in Trs formation [9], which are thought to be produced in about an equal ratio as Dics following ionizing radiation exposure [19, 20]. Because cells with Trs are mitotically stable, an increase in Trs induced by a CT examination could be hidden in the frequency of Trs formation due to various confounding factors. On the other hand, the frequency of Trs formation was higher than that of Dics formation both before and after CT examination [9]. The features of these chromosome aberrations were observed similarly in this study.

Cells with Dics are unable to survive for a long time because those cells are mitotically unstable and unable to undergo repeated...
Table 2. Results of dicentric chromosome and translocation analyses

| Patient No. | Blood sampling<sup>a</sup> | DLP (mGy-cm) | Effective dose (mSv)<sup>b</sup> | Analysis of dicentrics (Dics) | Analysis of translocations (Trs) |
|-------------|-----------------------------|--------------|-------------------------------|-----------------------------|---------------------------------|
|             |                             |              |                               | Cell counts | Dics | Frequency<sup>d</sup> | Increment | Cell counts | Cell equivalent<sup>c</sup> | Trs | Frequency<sup>d</sup> | Increment |
| 1           | 1B                           | 2679.70      | 46.30                         | 2009        | 7    | 0.348               | 0.1       | 5120        | 2021                | 157 | 7.767               | 0.799     |
|             | 1A                           | 2008         |                                | 2008        | 9    | 0.448               |           | 175         | 2043                | 175 | 8.566               |           |
|             | 2B                           | 3695.85      | 57.88                         | 2005        | 6    | 0.299               | −0.050    | 1120        | 2018                | 122 | 6.045               | 2.382     |
|             | 2A                           | 2010         | 5                             |             | 5    | 0.249               |           | 1100        | 2017                | 170 | 8.427               |           |
|             | 3B                           | 3291.20      | 57.17                         | 2009        | 7    | 0.348               | 0.101     | 1149        | 2033                | 166 | 8.166               | 0.188     |
|             | 3A                           | 2003         | 9                             |             | 9    | 0.449               |           | 1124        | 2023                | 169 | 8.354               |           |
| 2           | 1B                           | 1682.30      | 30.82                         | 2015        | 11   | 0.546               | 0.201     | 5211        | 2030                | 35  | 1.724               | 1.052     |
|             | 1A                           | 2007         | 15                            |             | 15   | 0.747               |           | 179         | 2018                | 56  | 2.776               |           |
|             | 2B                           | 4354.12      | 75.22                         | 2012        | 9    | 0.447               | 0.15      | 139         | 2002                | 39  | 1.948               | 1.504     |
|             | 2A                           | 2009         | 12                            |             | 12   | 0.597               |           | 131         | 1999                | 69  | 3.452               |           |
|             | 3B                           | 4222.08      | 68.25                         | 2008        | 10   | 0.498               | 0.101     | 132         | 1999                | 62  | 3.101               | −0.199    |
|             | 3A                           | 2005         | 12                            |             | 12   | 0.599               |           | 130         | 1998                | 58  | 2.902               |           |
| 3           | 1B                           | 5856.00<sup>b</sup> | 54.40               | 2011        | 9    | 0.448               | 0.2       | 5182        | 2046                | 155 | 7.577               | 2.199     |
|             | 1A                           | 2005         | 13                            |             | 13   | 0.648               |           | 234         | 2066                | 202 | 9.776               |           |
|             | 2B                           | 4119.80      | 69.47                         | 2006        | 8    | 0.399               | 0.149     | 215         | 2059                | 198 | 9.617               | −1.174    |
|             | 2A                           | 2008         | 11                            |             | 11   | 0.548               |           | 130         | 2025                | 171 | 8.443               |           |
|             | 3B                           | 3321.70      | 60.16                         | 2007        | 12   | 0.598               | 0.148     | 105         | 2015                | 169 | 8.385               | 1.8       |
|             | 3A                           | 2012         | 15                            |             | 15   | 0.746               |           | 521         | 2180                | 222 | 10.185              |           |
| 4           | 1B                           | 3319.80      | 61.83                         | 2007        | 8    | 0.399               | 0.045     | 5121        | 2022                | 40  | 1.979               | 1.293     |
|             | 1A                           | 2025         | 9                             |             | 9    | 0.444               |           | 264         | 2078                | 68  | 3.272               |           |
|             | 2B                           | 3046.10      | 50.67                         | 2003        | 6    | 0.3                 | 0.1       | 272         | 2081                | 59  | 2.835               | 0.435     |
|             | 2A                           | 2001         | 8                             |             | 8    | 0.4                 |           | 154         | 2232                | 73  | 3.27                |           |
|             | 3B                           | 2635.20      | 46.72                         | 2000        | 7    | 0.35                | 0.099     | 590         | 2135                | 61  | 2.858               | 0.207     |
|             | 3A                           | 2006         | 9                             |             | 9    | 0.449               |           | 589         | 2088                | 64  | 3.065               |           |
| 5           | 1B                           | 2448.40      | 46.22                         | 2008        | 16   | 0.797               | 0.098     | 5520        | 2179                | 101 | 4.635               | −0.368    |
|             | 1A                           | 2012         | 18                            |             | 18   | 0.895               |           | 402         | 2133                | 91  | 4.267               |           |
|             | 2B                           | 1990.50      | 37.25                         | 2005        | 16   | 0.798               | 0.15      | 204         | 2054                | 99  | 4.819               | 0.656     |
|             | 2A                           | 2004         | 19                            |             | 19   | 0.948               |           | 228         | 2064                | 113 | 5.475               |           |
|             | 3B                           | 1704.20      | 32.46                         | 2002        | 20   | 0.999               | 0.151     | 141         | 2030                | 84  | 4.139               | −1.375    |
|             | 3A                           | 2000         | 23                            |             | 23   | 1.15                |           | 132         | 2026                | 56  | 2.764               |           |

(Continued)
Table 2. Continued

| Patient No | Blood sampling<sup>a</sup> | DLP (mGy·cm<sup>−1</sup>) | Effective dose (mSv<sup>b</sup>) | Analysis of dicentrics (Dics) | Analysis of translocations (Trs) |
|------------|-----------------------------|---------------------------|---------------------------------|------------------------------|---------------------------------|
|            |                             |                           |                                 | Cell counts | Dics | Frequency<sup>c</sup> | Increment | Cell counts | Cell equivalent<sup>e</sup> | Trs | Frequency<sup>d</sup> | Increment |
| 6          | 1B                           | 1248.00                   | 27.06                           | 2001         | 5    | 0.25                | 0.149      | $109         | 990         | 95 | 4.773             | −0.632     |
|            | 1A                           | 2005                      | 0.399                           | 2003         | 8    | 0.25                | 0.246      | $145         | 2004         | 83 | 4.141             |           |
|            | 2B                           | 2059.93                   | 35.95                           | 2015         | 10   | 0.496               | −0.632     | $168         | 2013         | 112 | 5.563             |           |
|            | 2A                           | 1656.50                   | 32.73                           | 2002         | 4    | 0.2                 | −0.050     | $210         | 2030         | 48 | 2.365             | 1.178      |
|            | 3A                           | 2001                      | 0.15                            |              |      |                     |            | $216         | 2032         | 72 | 3.543             |           |
| 7          | 1B                           | 3368.50                   | 62.61                           | 2004         | 6    | 0.299               | 0.1        | $192         | 2050         | 89 | 4.342             | 3.185      |
|            | 1A                           | 3064.30                   | 56.59                           | 2003         | 8    | 0.399               | 0.049      | $115         | 2019         | 152 | 7.527             |           |
|            | 2B                           | 2405.70                   | 46.98                           | 2006         | 4    | 0.199               | 0.095      | $162         | 2038         | 171 | 8.391             |           |
|            | 3A                           | 2405.70                   | 46.98                           | 2004         | 4    | 0.195               | 0.095      | $222         | 2062         | 146 | 7.082             |           |
| 8          | 1B                           | 3265.60                   | 63.54                           | 2000         | 17   | 0.85                | 0.15       | $112         | 1991         | 681 | 34.197            | 0.735      |
|            | 1A                           | 2000                      | 1                               | 2001         | 14   | 0.7                 | 0.1        | $144         | 2004         | 700 | 34.932            |           |
|            | 2B                           | 3113.00                   | 64.21                           | 2002         | 16   | 0.8                 | 0.1        | $232         | 2038         | 589 | 28.898            | 3.414      |
|            | 2A                           | 2757.60                   | 56.96                           | 2005         | 12   | 0.599               | 0.15       | $137         | 2001         | 730 | 36.479            | 5.925      |
|            | 3A                           | 2003                      | 0.749                           |              |      |                     |            | $194         | 2023         | 858 | 42.404            |           |

<sup>a</sup> Blood sampling either before (B) or after (A) the first (1), second (2) third (3) CT examination.

<sup>b</sup> CTDI was defined using 16 cm phantom. All other CTDI were defined using 32 cm phantom.

<sup>c</sup> Estimated effective radiation dose (whole body exposure dose) according to ICRP 103 using WAZA-ARI.

<sup>d</sup> Frequency in 100 cells.

<sup>e</sup> Cell counts were converted to the equivalent number of cells as described in the Materials and Methods section.
Chromosome aberration in consecutive CT scans

Fig. 1 Frequency of Dics formation and changes in frequency with three consecutive CT examinations. (A) Analysis of Dics formation before and after each of three CT examinations. The light colored bar indicates the frequency before CT and the dark colored bar indicates the frequency after CT. 1st-B, 2nd-B and 3rd-B: before 1st CT, 2nd CT and 3rd CT, respectively. 1st-A, 2nd-A and 3rd-A: after 1st CT, 2nd CT and 3rd CT, respectively. (B) Comparison of the frequency of Dics formation before and after each CT examination. The frequency of Dics formation increased significantly after each CT examination: (a) 1st CT examination, \( P = 0.0003 \); (b) 2nd CT examination, \( P = 0.008 \); (c) 3rd CT examination, \( P = 0.007 \). (C) Comparison of the frequency of Dics formation before the 1st CT and after the 3rd CT. No significant difference was found \( (P > 0.05) \).
Fig. 2 Frequency of Trs formation and changes in frequency with three consecutive CT examinations. (A) Analysis of Trs formation before and after each of three CT examinations. The light colored bar indicates the frequency of Trs formation before CT and the dark colored bar indicates the frequencies after CT. 1st-B, 2nd-B and 3rd-B: before 1st CT, 2nd CT and 3rd CT, respectively. 1st-A, 2nd-A and 3rd-A: after 1st CT, 2nd CT and 3rd CT, respectively. CE = cell number converted to the equivalent of 100 cells. (B) Comparison of the frequency of Trs formation before and after each CT examination. No significant difference was found in the frequency of Trs formation in each CT examination. (C) Comparison of the frequency of Trs formation before the 1st CT and after the 3rd CT. No significant difference was found (P > 0.05).
Previous studies revealed DNA DSBs using the Dics analysis or γ-H2AX foci staining following CT examination. The frequency of Dics formation increased transiently following a CT examination, and then decreased, and a cumulative increase in the frequency of Dics formation by three consecutive CT examinations was not observed. Exceptionally, those two factors were not correlated in second and third CT examination in patient 1 (Table 2). We suspect that the difference in CTDIvol due to different parameters at CT examination was a cause.

Shi et al. analyzed chromosomal aberrations, dicentric and ring chromosomes in patients with ischemic heart disease before and after a CT scan procedure [29]. Similar to our results, they observed a significant increase in the number of dicentrics and rings that changes in a time-dependent manner after their formation, which depend on types of aberrations.

Furthermore, because the data may contain uncertainties concerning the estimation of dosimetry and the limited number of cases, further study is needed to generalize the conclusions in the present study.

**Supplementary Data**

Supplementary data is available at *Journal of Radiation Research* online.

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CONFLICT OF INTEREST

None declared.

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